A Comparison of Three Rapid Chlamydial Tests in Pregnant and Nonpregnant Women

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Background. The purpose of this investigation was to evaluate the performance characteristics of three rapid immunoassay tests to detect *Chlamydia trachomatis* cervicitis.
Methods. Endocervical samples from 506 women were evaluated using the three rapid immunoassay tests and the results were compared with those obtained by an endocervical chlamydial culture.
Results. The prevalence of *C trachomatis* cervicitis was 9.3%. Overall sensitivity and specificity of the Abbott TestPack Chlamydia test were 66.0% and 99.8%, respectively, of the Kodak Surecell Chlamydia test were 85.1% and 99.3%, respectively, and of the Unipath Clearview Chlamydia test were 85.1% and 98.5%, respectively. Pregnancy did not affect test specificity, but did influence sensitivity. The tests ranged from 5% to 22% less sensitive in nonpregnant women.
Conclusions. The results of the investigation establish that the Clearview and Surecell chlamydial immunoassay tests performed well, particularly for pregnant women.
Key words. Chlamydia trachomatis; immunoenzyme technics; cervicitis.
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The use of immunoassay tests in the physician office laboratory now permits rapid and comparatively inexpensive detection of microorganisms. The relatively simplistic immunoassay technology enables appropriately trained office personnel to reliably conduct these tests.[1] Assay tests for office laboratory use are particularly appealing when an expensive, complex, labor-intensive culture test is the alternative option. Add to the preceding factors the difficulties in transporting these cultures to a reference laboratory and the presence of a temperature-labile organism, and the need for an on-site immediate assay becomes even more obvious. Irrespective of admirable qualities, however, the immunoassay test must produce accurate results.

The clinician's ability to detect cervical *Chlamydia trachomatis* infection solely by symptoms or physical findings is poor. Laboratory testing thus becomes critical. The *C trachomatis* culture is considered the criterion standard of comparison. The culture method is easily influenced by many factors, including several factors that are common to immunoassay tests. Both tests are ultimately dependent on the collection of an adequate specimen of endocervical columnar epithelial cells that are infected with the obligate intracellular *C trachomatis* organism.[2] Ectocervical mucus and debris may affect both culture and immunoassay tests.[3] Excessive cellular material or other microorganisms or agents may be toxic to culture McCoy cells and may impede immunoassay processing.[4] The detection of *C trachomatis* also varies proportionately with the number of organisms contained in a specimen. Older women with asymptomatic cervical chlamydial infections often have fewer organisms present than symptomatic young women. The culture would be more sensitive than the antigen assay in cases of low-level infection.[5]

Yet, the sensitivity of a culture of a single endocervical specimen has been reported[3,6] to be only 67% to 77% rather than 100%. Multiple swabs increase the positivity rate of detection.[3] Culture toxicity due to contamination decreases as the number of specimen collections increases.[3] Elevated serum antibody titers to *C trachomatis,* recent patient treatment with antibiotics, culture inoculation delay, and improper thermal transport or storage conditions affect culture outcome.[5] Chlamydial cell culture technique has been refined and improved by the use of direct fluorescent antibody confirmation, cycloheximide pretreatment of McCoy

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cells, and the use of shell vials and nontoxic collection materials.[2]

Similarly, immunoassay systems have been modified and improved. The purpose of the investigation was to evaluate immunoassay performance characteristics for two rapid chlamydial enzyme immunoassay (EIA) kits previously evaluated but subsequently modified.[1] Also, a third new solid-phase sandwich immunoassay chlamydial kit, not previously available, was compared with the other two kits.

Methods

Patient Population

All women between the ages of 18 and 35 years seeking routine gynecologic medical care at one of the study sites were asked to participate in the investigation. Subjects were recruited from the Medical College of Georgia Student Health Clinic, the Richmond County Health Department Family Planning and Prenatal Clinics, and Planned Parenthood, all located in Augusta, Georgia. Other subjects were enrolled by the Rural Outreach Maternal and Infant Program practicing within 16 counties surrounding Augusta, Georgia.

Inclusion criteria were that the woman be 18 to 35 years of age and voluntarily agree to participate in the investigation. Exclusion criteria were the use of antibiotics within the previous 4 weeks or douching within 24 hours before the examination.

Laboratory Methods

CHLAMYDIAL CULTURE

After specimen collection, swabs were placed in 2-S P containing transport medium and held at 4°C pending delivery the same day. On receipt in the laboratory, the tubes were mixed vigorously, and 0.25 mL of the specimen was inoculated into dram vials containing coverslips of light-density McCoy cells.

Following inoculation, all vials were centrifuged at 3000 × g for 60 minutes at 33°C to 35°C. After centrifugation, vials were rinsed with phosphate-buffered saline, refilled with minimum essential medium (MEM) containing 1.0 μg/mL cycloheximide, and incubated for 48 hours at 35°C. All vials were then fixed with cold acetone and stained with a fluorescein-conjugated monoclonal antibody against a chlamydial major outer membrane protein. Coverslips were examined using a 100-watt epifluorescence microscope at a magnification of ×250. Positive cultures were graded 1+ to 4+ depending on the number of inclusions seen. Coverslips exhibiting signs of toxicity were excluded from further data analysis.

DISCORDANT CULTURE RESOLUTIONS

A direct fluorescent antibody test method was used to resolve discrepancies when the immunoassay was positive and the culture was negative. Vials of specimens that had been frozen in 2-S P were thawed, mixed, transferred to microfuge tubes, and centrifuged at 10,000 × g for 10 minutes. Supernatant fluid was discarded, and the pellet was resuspended in 50 μL of phosphate-buffered saline. Ten microliters of the resuspended pellet was placed onto Teflon-coated microscope slides. The slides were air dried, fixed with cold methanol, and stained with a *Chlamydia trachomatis* fluorescein-conjugated reagent. Slides exhibiting microscopic fluorescent elementary bodies were confirmed at a magnification of ×1000 and considered to represent a true-positive specimen (ie, a false-negative culture result).

IMMUNOASSAYS

The Abbott TestPack Chlamydia (Abbott Laboratories, North Chicago, Ill), Kodak Surecell Chlamydia (Eastman Kodak Co, Rochester, NY), and Unipath Clearview Chlamydia (Unipath Co, Mountain View, Calif) immunoassay tests were performed according to the manufacturers' specifications by one medical technologist to control for variability. The medical technologist, located in a separate laboratory, was blinded to culture results.

Testing was conducted the day of collection on endocervical swab specimens. The collection swabs used were those provided in the respective manufacturers' kits. Positive controls were performed daily. Kodak EIA positivity was defined as the appearance of a substantially darker red color in sample well No. 2 compared with negative well No. 1. Abbott EIA positivity was defined as any light to deep red color on the vertical line against a yellow background. A positive sign (+) indicated the presence of chlamydial antigen. Clearview immunoassay positivity was defined as the presence of a horizontal line in both the control window and the result window.

Study Design

Clinicians obtained standardized histories from the patients, secured informed consent, and performed brief anogenital examinations before collecting specimens. Following optional endocervical culturing for *Neisseria gonorrhoeae* and sampling for Papanicolaou smears, four swabs were used to collect endocervical samples for chlamydial testing. The first Dacron swab was used for chla-

Table 1. Comparison of Clearview, Surecell, and TestPack with Culture for the Detection of Endocervical *Chlamydia trachomatis* Infection in 506 Women with a 9.3% Prevalence of Infection

Test Performance	Clearview*	Surecell†	TestPack‡
Sensitivity, % (95% CI)	85.1 (71.7–93.8)	85.1 (71.7–93.8)	66.0 (50.7–79.1)
Specificity, % (95% CI)	98.5 (96.9–99.4)	99.3 (98.1–99.9)	99.8 (98.9–100.0)
Positive predictive value, %	85.1	93.0	96.8
Negative predictive value, %	98.5	98.5	96.6

**Clearview Chlamydia, Unipath Co, Mountain View, Calif.*
†Surecell Chlamydia, Eastman Kodak Co, Rochester, NY.
‡TestPack Chlamydia, Abbott Laboratories, North Chicago, Ill.

mydial culture processing. The sequence in which each of the swabs from the three immunoassay test kits was used was consecutively alternated for each patient to allow for inconsistencies in methods of specimen collection. All specimens were collected during one patient visit and were kept refrigerated until delivery to the respective laboratories.

Statistical Analysis

The chi-square test or Fisher's exact test was used to compare each test's performance with different subject groups. Exact confidence intervals for the estimated sensitivities and specificities were calculated by the methods of Fisher and Yates. McNemar's test with Bonferroni's adjustment for multiple comparisons was used for comparisons between the immunoassay test performance data.

Results

A total of 527 patient specimens were collected from March 1991 to May 1991, and tested for *C trachomatis*. Complete data are available for 506 subjects. Sixteen chlamydial cultures that displayed cytotoxic effects and two missing enzyme immunoassay swabs were excluded from the calculations. All specimens taken from three subjects were lost. Forty-seven patients were identified as having positive chlamydial cultures. The overall prevalence of *C trachomatis* cervicitis was 9.3%. The prevalence of infection was 14.6% in the pregnant subject group and 6.9% in the nonpregnant group ($P = .013$).

Patient demographics showed that the mean age was 22.7 years. One third (33.8%) of the subjects were pregnant. A history of previous chlamydial infection was confirmed by 15% of the subjects. The significance of a previous chlamydial infection was evaluated. Only 3.0% of the women who reported a history of prior chlamydial infection were confirmed to have *C trachomatis* cervicitis at the time of the present study, whereas 10.3% of women without a previous history of *C trachomatis* infection were identified as currently having chlamydial cervicitis. The majority (63%) of subjects reported no prior history of sexually transmitted disease. Most nonpregnant subjects (57%) used oral contraceptive agents, and 11% used barrier methods of contraception (condom or diaphragm). Most (72.7%) of the women were asymptomatic.

The majority (95.6%) of women with a wet-mount preparation demonstrating less than 10 leukocytes per high power field were not infected with *C trachomatis*. However, 88.2% of women confirmed to be infected with *C trachomatis* had more than 10 leukocytes per high power field by vaginal wet-mount preparation examination ($P = .18$). None of the women who used barrier contraceptive methods (condom or diaphragm) were infected with *C trachomatis*. Yet 10.1% of the women who used nonbarrier contraception (oral contraceptive agents) or no contraception were identified as having chlamydial cervicitis ($P = .04$).

The three immunoassay test performance results, when compared with the *C trachomatis* culture, are recorded in Table 1. The Clearview and Surecell test sensitivities were equal at 85.1%. The sensitivity of the TestPack assay was 66.0%. The differences between the Clearview and Surecell sensitivities compared with the TestPack sensitivity were statistically significant, $P < .05$. The specificities were comparable and ranged from 98.5% to 99.8%.

The Surecell EIA performed best in the pregnant population group, with a sensitivity of 95.8% and a specificity of 99.3%. The Surecell sensitivity and specificity for nonpregnant women were 73.9% and 99.4%, respectively. The Clearview immunoassay performed best in the nonpregnant group with a sensitivity of 82.6% and specificity of 98.7%. The Clearview sensitivity and specificity for pregnant women were 87.5% and 97.0%, respectively. The TestPack specificities of 100% and 99.7% were best in both the pregnant and nonpregnant groups, respectively. TestPack sensitivities for the pregnant and nonpregnant women were 75.0% and 56.5%, respectively.

Discussion

The results of this investigation demonstrate that two of the three immunoassay tests evaluated, Clearview Chlamydia and Surecell Chlamydia, performed well. Sensitivities for both test kits were 85.1%, and specificities were 98.5% and 99.3%, respectively, in a study population with an overall *C trachomatis* infection prevalence of 9.3%.

The TestPack Chlamydia EIA did not perform as well as the other immunoassay tests. The test sensitivity of 66.0% indicates that the test failed to detect disease in about one fifth of the women who were infected and who were correctly identified by the other two immunoassay tests. Furthermore, the TestPack performance in this investigation does not reflect the sensitivity that was reported in the package insert of 80.5% in a population with an infection prevalence of 12.9%.

All three immunoassay tests demonstrated a greater sensitivity in the pregnant population. Smith et al[7] also reported 20% to 25% greater sensitivity of two antigen detection tests in a population of pregnant women when compared with the test's sensitivity in nonpregnant women. The specific cervical epithelial changes of pregnancy may account for the test performance variability. More chlamydial inclusion forming units are microscopically visible in pregnant patients compared with nonpregnant patients.[7]

The test sensitivity confidence intervals (Table 1) were wide, owing to the small study size and moderate chlamydial prevalence. There was a statistically significant difference, however, between the results obtained using the TestPack Chlamydia and those obtained using the other two immunoassay tests.

There have been few comparison studies of rapid immunoassays. The variations in test performances reported in the literature are due to differences in the criterion standard, test processing methods, and prevalence of *C trachomatis* in the population. The performance data for the Clearview Chlamydia test approximated data reported by Young et al.[8] In an 8.8% prevalence of chlamydial infection, the Clearview Chlamydia sensitivity was 85.7% and specificity was 99.1%. In the study of 478 women, cell cultures were frozen before processing, and chlamydial confirmation was by iodine staining. Both practices can adversely influence the detection of *C trachomatis*. Fewer inclusion forming units are noted following freezing, and comparatively fewer are identified with iodine staining as opposed to immunofluorescence confirmation techniques. The methodologic impact may contribute to false-negative cultures and a resultant overestimated test sensitivity. In a smaller study of 376 women, Arumainayagam et al[9] reported that the Clearview Chlamydia had a sensitivity of 93.5% and a specificity of 99%. Swab collection order was randomized, however; therefore, the first swab was not always used for culture, which is the optimal study approach.

Several clinical investigations evaluating TestPack Chlamydia have been published that failed to use a culture criterion standard and instead compared TestPack Chlamydia with another more complex enzyme immunoassay test not suitable for the office laboratory.[10,11] In a large multisite study, however, Coleman et al[12] compared the performance of TestPack with that of culture to detect *C trachomatis*. The sensitivity of TestPack Chlamydia results was 72.9% and the specificity was 97.4% when specimens processed in less than 48 hours were used. The overall prevalence of infection in that study population was 11.6%. Yet, this investigation also used frozen culture specimens and collected specimens for culture and EIA in a random order. All specimens for TestPack assay were blindly tested at the manufacturer's corporate laboratories. An operator-proficiency bias may have resulted from this practice. Reichart[13] compared results obtained using TestPack with those obtained using culture to detect *C trachomatis* in 285 women with an identified 15% prevalence of infection. The TestPack sensitivity was 66% and the specificity was 99%, the same results obtained in the current investigation. The specimen for culture was always obtained last, however, and all culture specimens were frozen before inoculation. While the results were the same as those reported herein, the TestPack kit used by Reichart was not the modified version evaluated in this investigation.

In only one[1] of the previously cited investigations were the immunoassay tests actually performed in family practice or office-based laboratories. Tests intended for use in the physician's office laboratory should be evaluated in a comparable setting, not in a reference, corporate, or hospital laboratory. Thus, pre- and postanalytical factors that would commonly influence test performance are allowed to occur, and actual clinical performance may be more accurately predicted.

The chlamydial immunoassay tests evaluated in this study use similar EIA methodologies, but each manufacturer has incorporated certain noteworthy features. The TestPack Chlamydia test featured a yellow test-well background that contrasted nicely with the result indicator of a red plus or minus sign. The indicators were also the easiest test endpoints to interpret. The small removable reagent holder, specifically designed to hold only those reagents that require refrigeration, was a useful addition. The plastic extraction tubes were not squeezable, however, making it difficult to extract fluid from the hard, smooth specimen-collection swabs. The TestPack processing time was approximately 18 to 20 minutes, and the hands-on time was 3 to 5 minutes. (Note: Four months

following completion of this investigation, the TestPack Chlamydia was recalled by the manufacturer, and product distribution was terminated owing to evidence of suboptimal test performance.)

The Surecell Chlamydia test required the shortest processing time (11 to 13 minutes). The extraction process was facilitated by pliable extraction tubes. Positive and negative quality-control systems were incorporated in the kit. The test endpoint indicator system was also fairly easy to interpret. The Surecell test has been approved by the Food and Drug Administration for use with men, but the TestPack and Clearview have no current product indication. Otherwise, the test required more laboratorian hands-on time (5 to 7 minutes), which may be a determining factor in choosing a test for use in a busy office practice with limited personnel. The specimen fluid available following the addition of reagents was frequently insufficient to fill all three test wells as required.

The simplicity of the Clearview Chlamydia immunoassay test was apparent. The test requires only one reagent and one extraction step. There are no wash steps. The unique test contains the necessary antibodies already positioned and impregnated within a pad that permits the capillary migration of the specimen from the sample window toward the result window. Therefore, the usual series of steps conducted by the laboratorian are performed automatically during the specimen migration through the pad. The specimen could be dropped into the test sample window and the laboratorian could leave to perform other duties. In other words, the test featured the least amount of hands-on time required (1 to 2 minutes). Test completion was indicated by a line in the control window when the specimen had migrated to the end of the pad. The Clearview test required a heat block for extraction and required the longest processing time (25 to 30 minutes). Finally, weak positive results were more difficult to interpret because of a rather faintly visible line that could be easily overlooked.

The modified Surecell and TestPack tests both demonstrated improved performance when compared with previously published results obtained using earlier versions of these tests.[1] In a similar population, the TestPack sensitivity increased from the previously reported 51.7% to 66.0%. The Surecell previously reported sensitivity of 76.7% improved to 85.1% in the current investigation. Of note, the prevalence of *C trachomatis* infection in the previous comparative investigation was 12.0%, actually greater than the 9.3% reported here. The current study included a greater percentage of pregnant women, which may have affected performance outcomes.

In conclusion, rapid chlamydial immunoassay tests do not perform equally well. The evolution of immunoassay tests has been characterized, however, by a high specificity, a gradually improving sensitivity, and significantly easier test processing. Some current chlamydial immunoassay tests are better accommodated in the busy physician office laboratory and are sufficiently sensitive and specific for routine use in high-risk population groups. Finally, rapid immunoassay tests are more accurate at identifying *C trachomatis* cervical infection in pregnant women than in nonpregnant women.

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