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Multicenter Comparative Evaluation of Two Rapid Microscopic Methods and Culture for Detection of *Chlamydia trachomatis* in Patient Specimens

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Four hundred and seventy-three men and women at high risk for sexually transmitted disease were tested for the presence of *Chlamydia trachomatis* in the urethra or the endocervix. Four groups were involved in this multicenter study of two direct fluorescent-antibody microscopy tests, Kallestad Pathfinder and Syva Microtrak, compared with culture techniques. Results from the test sites indicated that there was no significant difference overall in the sensitivity and specificity of the two test kits. However, there was some interlaboratory variation seen in the sensitivity of the microscopy, but little difference in the specificity. Either kit could be an effective screening method for *C. trachomatis* in high-risk populations.

There are a number of rapid methods available for detection of *Chlamydia trachomatis*. They include both direct fluorescent-antibody microscopy (DFA) and enzyme immunoassay (EIA). The Syva DFA (Syva, Inc., Palo Alto, Calif.) method has been evaluated by a number of investigators (8, 14–17). With the exception of the original studies (14, 15), the reports have indicated test sensitivity in the 70 to 80% range. Ryan et al. (12) compared an EIA (Chlamydiazyme) (Abbott Laboratories, North Chicago, Ill.) with culture on 1,074 patients in three risk groups. The rapid EIA proved to be a highly specific and sensitive procedure for detection of *C. trachomatis* in genital specimens from a high-risk female population and symptomatic males.

The present study was performed in five centers on a high-risk population and compared two DFA tests, Kallestad Pathfinder (Kallestad, Winona, Minn.) and Syva MicroTrak with culture.

MATERIALS AND METHODS

Study centers. This collaborative study was carried out in five centers: the University of Connecticut Health Center (John Dempsey Hospital), Farmington; the Centers for Disease Control (CDC), Atlanta, Ga.; the Denver Public Health Department, Denver, Colo.; Hennepin County Medical Center, Minneapolis, Minn.; and the University of Iceland, Reykjavik. The University of Connecticut-University of Iceland study was a joint effort. All study centers used essentially the same cultural procedures for *C. trachomatis* and performed both DFA tests as specified by the manufacturers.

Patient population. The patient populations studied were at high risk for sexually transmitted diseases (STDs). Four hundred and seventy-three male and female patients attending STD clinics with genitourinary symptoms and asymptomatic contacts of partners with STD were enrolled.

Specimens. Two endocervical or urethral swabs were collected from each patient. If there was visible discharge, the discharge was either collected for *Neisseria gonorrhoeae* culture or wiped from the orifice. Care was taken to ensure that culture collection techniques were sufficiently aggressive to ensure adequate mucosal epithelial cells in the specimen. The first swab taken was placed in 2 ml of *Chlamydia* transport medium (0.2 M sucrose phosphate buffer) and cultured. The second swab was used to prepare the slides for DFA microscopy. The order in which the slides were prepared was randomized.

Culture. All specimens were cultured by accepted methods (11) with cycloheximide-treated McCoy cells. All cultures were stained at 48 h with the Syva fluorescent-antibody culture confirmation reagent. Those specimens containing >1 inclusion were considered positive. Negative cultures were passed once and restained.

DFA. Specimens for DFA were collected on the dacron swabs provided in each DFA kit. The swabs for both kits are essentially identical. The swab was rolled over the circular scribed area of the glass slides provided and appropriately labelled. Kallestad slides were fixed with methanol, and Syva slides were fixed with acetone. Control slides provided were similarly processed. One drop (approximately 30 μl) of either antibody reagent was added to each well, and the slides were incubated for 15 min at room temperature in a moist chamber. The slides were rinsed with distilled deionized water for 10 to 15 s and air dried. One drop of the mounting fluid provided was added to the well, and then a cover slip was carefully placed over the well. The entire circumscribed area on the slide was screened with an epifluorescence microscope at a total magnification of 400 to 630× under oil. Positive slides were confirmed by using a 1,000× oil immersion objective. Slides with no cellular material present were rejected as representing inadequate specimen collection.

Statistical tests. Sensitivity, specificity, and predictive

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TABLE 1. Comparison of two DFA tests for *C. trachomatis* with culture for 473 high-risk male and female patients

Chlamydia culture result	No. of results by DFA[a]			
	Positive		Negative	
	KP	SM	KP	SM
Positive	117	108	23	32
Negative	9	10	324	323

[a] KP, Kallestad Pathfinder; SM, Syva MicroTrak.

values were determined by the method of Galen et al. (5). Upper and lower limits of sensitivity and specificity of the combined results for high-risk men and women were calculated by William Longley, Marion Laboratories, Kansas City, Mo.

RESULTS

Table 1 presents the combined results for 473 male and female patients when two DFA tests were compared with culture for detection of *C. trachomatis*. Included in Table 1 are 220 high-risk male patients. For this population, the sensitivity of Pathfinder and MicroTrak was 84.1 and 75.4% and the specificity was 98.7 and 98.0%, respectively. The incidence of disease in this population was 31.2%.

Table 1 also includes similar data for a high-risk female population comprising 253 symptomatic women or contacts of partners with *Chlamydia* infection. The prevalence of disease was slightly lower (27.1%) than in male patients. Sensitivity was 83.1 and 78.9% for Pathfinder and Micro Trak, respectively, and the specificity of the two kits was identical (96.2%).

When female and male patients were combined (Table 1), the overall disease prevalence was 29.5%. For the Kallestad kit, in 473 patients, the 95% confidence limits for sensitivity were 78.7 to 90.6%. For the Syva kit, the 95% confidence limits were 67.7 to 82.0%. There was no statistically significant difference in the sensitivity of the two kits, although the Syva kit had nine more false-negative results than the Kallestad kit. Similarly, there was no statistically significant difference in specificity for Pathfinder and MicroTrak (97.3 and 97.0%, respectively).

Table 2 is a compilation of the test results from the four sites. The CDC, with the exception of one male patient, tested only high-risk females. All other sites tested patients of both sexes. The sensitivity for all sites ranged from 66.7 to 100% and from 68.4 to 100% for Pathfinder and MicroTrak, respectively. The specificity of both kits was approximately 90% or greater. Prevalence ranged from a low of 6.5% among males at Hennepin County Medical Center to 54.3% among males in Iceland.

DISCUSSION

A number of rapid tests are available for detection of *C. trachomatis* in clinical specimens. These tests have the potential to replace the technically difficult and time-consuming traditional culture method and, with few exceptions, have used either DFA microscopy or EIA technology. As pointed out in many reports, there are advantages and disadvantages with each method. While the EIA is easier to perform and has a quantitative endpoint, it is less amenable than DFA to single-specimen processing. The significant questions to ask, however, are not necessarily methodological. They include: (i) Are the rapid tests applicable to all populations potentially at risk of chlamydial infections? (ii) Are there statistically significant differences in the performance characteristics of rapid tests? (iii) What is the role of *Chlamydia* culture in routine diagnosis of infection?

The predictive value of a positive or negative result in this study was similar with both rapid methods. It is recognized that while disease prevalence does not alter the sensitivity and specificity of a method, predictive value decreases with prevalence (2). For example, the study by Ryan et al. (12) compared a number of risk populations, among them high-risk symptomatic women and low-risk asymptomatic women. While the sensitivity and specificity were similar for both groups (91.3 and 89.3% versus 95.0 and 93.2%, respectively), the predictability of a positive test result decreased from 91.3% in the high-risk group (prevalence, 35.9%) to 44.6% in the low-risk group (prevalence, 5.8%). Both kits appear to be excellent screening tests for a high-risk population, but successful application to a low-risk population cannot be assumed.

An early report on MicroTrak (15) claimed a sensitivity of approximately 93%. Few investigators have been able to duplicate these results. In the present study, the overall sensitivity of MicroTrak was 77.1%. However, as can be seen in Table 2, the sensitivity and specificity of both DFA tests varied as a function of test site and population evaluated. The variability was extensive for both kits. For example, with the Pathfinder kit, the CDC and Hennepin County Medical Center showed a sensitivity of 77.8 and 66.7%, respectively, while on the same general population of patients, high-risk females, the Denver Group and the University of Connecticut-University of Iceland group showed a sensitivity of 90.3 and 90.0% for the same Kallestad DFA procedure. Similar variability was observed with the Syva kit.

The specificity of both DFA procedures was more uniform. Only the CDC group (high-risk females) and Hennepin County Medical Center (high-risk males) showed specificity of <98 to 100%. Several reasons may exist for such a discrepancy. If the cell culture technique is not optimal, then

TABLE 2. Individual-laboratory comparison of Pathfinder and MicroTrak DFA tests with culture in high-risk male and female patients

Study site	Risk group	No. of patients	Sensitivity[a] (%)		Specificity (%)		Prevalence (%)
			KP	SM	KP	SM	
CDC	Female	77[b]	77.8	72.2	89.8	89.8	23.3
Denver Public Health Department	Female	85	90.3	87.1	100	100	36.5
	Male	123	85.7	82.1	100	100	22.8
Hennepin County Medical Center	Female	56	66.7	75.0	99.7	97.7	21.1
	Male	30	100	100	92.9	89.3	6.5
University of Connecticut-University of Iceland	Female	35	90.0	70.0	100	100	28.9
	Male	67	81.6	68.4	100	100	54.3

[a] KP, Kallestad Pathfinder; SM, Syva MicroTrak.
[b] Does not include one high-risk male.

the rapid test will appear to be more sensitive but less specific. Schachter (13) indicated that the sensitivity of a single attempt to isolate *C. trachomatis* is unknown. However, it is unlikely that any of the current procedures are 100% sensitive. When patients are tested repeatedly, the sensitivity of a single test is approximately 90% for men with urethritis and 70 to 80% for women with cervicitis (13). In this study, attempts were made to standardize the parameters of sample collection and the culture technique, as well as the DFA procedure. All laboratories were experienced, so it was assumed that test variability would be minimal. Such was not the case, and there is no explanation for it except the expected inherent difference in patients, their extent of disease, and their organism load.

While it does not explain variability between test sites, there is evidence (12) that the second swab collected from a patient has fewer organisms than the first swab. In that study, 149 high-risk men and 90 high-risk women were evaluated for *C. trachomatis* by using the first swab for the direct EIA test and the second swab for culture. In men, sensitivity increased from 82.1 to 91.2% in the symptomatic group and from 47.8 to 64.7% in the asymptomatic group. A lesser increase in sensitivity was observed in the women, 90.9 to 93.8%. Reliance, then, on the second swab for the direct procedure probably does not ensure maximum sensitivity.

There is also a tendency to claim superiority of one kit over another based on a few percentage points of difference in sensitivity or specificity. The upper and lower limits of sensitivity and specificity of the kits suggest that such claims are without foundation unless there are either large numerical differences between kits or sufficient patient specimens so that upper and lower limits do not overlap. In the present study, there were fewer false-negative results with Pathfinder than with MicroTrak, but the difference was not significant.

While the culture of *Chlamydia* in cycloheximide-treated McCoy cells with DFA staining at 48 h is the most sensitive method of detection (13), it is not practical for most clinical laboratories. For high-risk populations, the DFA test becomes an attractive alternative. DFA is not as easy to perform or as cost effective as EIA, but DFA is more applicable to processing of a few specimens per day.

We suggest that the published studies on all rapid methods indicate that if the result is positive, it can be reported. However, if the test is negative, consideration should be given to culture techniques even if it must be sent to a reference laboratory. We suspect, from previous work (12), that the test is specific and sensitive in symptomatic men and both asymptomatic and symptomatic women. It is likely, however, that these tests are not suitable for asymptomatic male patients and sexually abused prepubertal girls (4). If any test is performed on this group, it probably should be a culture.

Several features of the Pathfinder test were noted. The fluorescent signal of the test was initially brighter than that of the MicroTrak but tended to fade more rapidly. This has been alleviated by modifications in the Pathfinder kit. More counterstain is used in the Pathfinder conjugate. Hence, the red-colored cells in the background are more evident. Some technologists prefer the increased red background, as it increases the contrast between negative cells and green elementary bodies; others do not.

The antibody used in both kits is monoclonal against epitopes in the major outer membrane protein of *C. trachomatis*. This differs from Chlamydiazyme, in which an antibody to lipopolysaccharide is used. Unlike the Boots-Celltech kit, which is a genus-specific polyclonal antibody, the Kallestad and Syva kits are species specific. A possible advantage of the Boots-Celltech product is the theoretical ability to detect *C. psittaci* var. TWAR, a reported cause of respiratory disease (6).

While this study has not evaluated the Kallestad method for specimens from infants with pneumonitis or conjunctivitis, other reports on Syva and EIA (3, 7, 9, 10) and our anecdotal experience suggest that the Kallestad DFA would be equally efficacious.

In summary, both DFA kits are comparable and should be considered for high-risk patients, particularly by laboratories without cell culture facilities.

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LITERATURE CITED

1. **Coudron, P. E., D. P. Fedorko, M. S. Dawson, L. G. Kaplowitz, R. R. Brookman, H. P. Dalton, and B. A. Davis.** 1986. Detection of *Chlamydia trachomatis* in genital specimens by the Microtrak direct specimen test. Am. J. Clin. Pathol. **85**:89–92.
2. **Ferraro, M. J. B., and L. J. Kunz.** 1982. Predictive value of microbiologic diagnostic tests, p. 248–267. *In* V. Lorian (ed.), Significance of medical microbiology in the care of patients, 2nd ed. The Williams & Wilkins Co., Baltimore.
3. **Friis, B., C. Kuo, S. P. Wang, C. H. Mordhorst, and J. R. Grayston.** 1984. Rapid diagnosis of *Chlamydia trachomatis* pneumonia in infants. Acta Pathol. Microbiol. Immunol. Scand. **92**:139–143.
4. **Fustov, C. D., and L. S. Neinstein.** 1987. Vaginal *Chlamydia trachomatis* prevalence in sexually abused prepubertal girls. Pediatrics **79**:235–238.
5. **Galen, R. S., and S. R. Gambino.** 1975. Beyond normality: the predictive value and efficiency of medical diagnosis. John Wiley & Sons, New York.
6. **Grayston, J. T., C. C. Kuo, S. P. Wang, and J. Altman.** 1986. A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. N. Engl. J. Med. **315**:161–168.
7. **Hammerschlag, M. R., J. E. Herrmann, P. Cox, M. Worku, R. Laux, and L. V. Howard.** 1985. Enzyme immunoassay for diagnosis of neonatal chlamydia conjunctivitis. J. Pediatr. **107**: 741–743.
8. **Lipkin, E. S., J. V. Moncada, M. A. Shafer, T. E. Wilson, and J. Schachter.** 1986. Comparison of monoclonal antibody stain and culture in diagnosing cervical *Chlamydia* infection. J. Clin. Microbiol. **23**:114–117.
9. **Paisley, J. W., B. A. Lauer, P. Melinkowich, B. A. Gitterman, D. J. Feiten, and S. Berman.** 1986. Rapid diagnosis of *Chlamydia trachomatis* pneumonia in infants by direct immunofluorescence microscopy of nasopharyngeal secretions. J. Pediatr. **109**:653–655.
10. **Rapoza, P. A., T. C. Quinn, L. A. Kiessling, R. Green, and H. R. Taylor.** 1986. Assessment of neonatal conjunctivitis with a direct immunofluorescent monoclonal antibody stain for *Chlamydia*. JAMA **255**:3369–3373.
11. **Ripa, K. V., and P. A. Mardh.** 1977. Cultivation of *Chlamydia trachomatis* in cyclohexamide treated McCoy cells. J. Clin. Microbiol. **6**:329–331.
12. **Ryan, R. W., I. Kwasnik, O. Steingrimsson, J. Gudmundsson, H. Thorarinsson, and R. C. Tilton.** 1986. Rapid detection of *Chlamydia trachomatis* by an enzyme immunoassay method. Diagn. Microbiol. Infect. Dis. **5**:225–234.
13. **Schachter, J.** 1984. Biology of *Chlamydia trachomatis*, p. 243–257. *In* K. K. Holmes (ed.), Sexually transmitted diseases. McGraw-Hill Book Co., New York.
14. **Stamm, W. E., H. R. Harrison, E. R. Alexander, L. D. Cles, M. R. Spence, and T. C. Quinn.** 1984. Diagnosis of *Chlamydia*

trachomatis by direct immunofluorescence staining of genital secretions. Ann. Intern. Med. **101**:638–641.

15. **Tam, M. R., W. E. Stamm, H. H. Handsfield, R. Stephens, C. C. Kuo, K. K. Holmes, K. Ditzenberger, M. Krieger, and R. C. Nowinski.** 1984. Culture independent diagnosis of *Chlamydia trachomatis* using monoclonal antibodies. N. Engl. J. Med. **310**: 1146–1150.
16. **Tjiam, K. H., R. V. W. van Eijk, B. Y. M. van Heijst, G. J. Tideman, T. van Joost, E. Stolz, and M. F. Michel.** 1985. Evaluation of the direct fluorescent antibody test for diagnosis of chlamydial infections. Eur. J. Clin. Microbiol. **4**:548–552.
17. **Uyeda, C. T., P. Welborn, N. Ellison-Birang, K. Shunk, and B. Tsaouse.** 1984. Rapid diagnosis of chlamydial infections with the Microtrak direct test. J. Clin. Microbiol. **20**:948–950.