JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1989, p. 1863–1866
0095-1137/89/081863-04$02.00/0

Vol. 27, No. 8

Cytobrush in Collection of Cervical Specimens for Detection of *Chlamydia trachomatis*

JEANNE MONCADA,[1] JULIUS SCHACHTER,[1]* MIRIAM SHIPP,[2] GAIL BOLAN,[2] AND JUDITH WILBER[3]

Department of Laboratory Medicine, University of California, San Francisco, California 94143,[1] and San Francisco City Clinic[2] and Department of Microbiology,[3] San Francisco Department of Public Health, San Francisco, California 94103

Received 3 March 1989/Accepted 8 May 1989

Methods of collecting endocervical samples for the detection of *Chlamydia trachomatis* were evaluated. We compared Calgiswab and cytobrush for isolation in cell culture and Dacron swab and cytobrush for direct fluorescent-antibody (DFA) testing for 632 females attending a sexually transmitted disease clinic. An additional specimen for enzyme immunoassay (EIA) was also collected. True-positives were identified as tissue culture positive and/or both DFA and EIA positive. Use of the cytobrush significantly improved the sensitivity of both the culture (69% with swab and 100% with cytobrush) and DFA testing (68% with swab and 85% with cytobrush). The EIA sensitivity was 85%. The specificity of each test was ≥98%. The cytobrush appears to be the superior method for the collection of cervical samples from nonpregnant women.

Chlamydia trachomatis has been recognized as an important pathogen in sexually active adults, with an estimated 4 million new cases occurring annually in the United States (20). In females, chlamydial infection often causes cervicitis and/or urethritis. It has also been implicated in bartholinitis, endometritis, and perihepatitis. It is a leading cause of acute salpingitis (1). Inapparent infections are common (17). Laboratory diagnostic tests are needed to identify these infections.

Tissue culture (TC) systems, particularly cycloheximide-treated McCoy cells, have become the "gold standard" for identifying *C. trachomatis* (13, 14). However, TC is both costly and time-consuming. New nonculture diagnostic methods provide easier, more rapid, and perhaps less expensive alternatives. The two most popular tests are antigen detection methods: a fluorescein-conjugated monoclonal antibody (direct fluorescent-antibody [DFA] test) (MicroTrak; Syva Co., Palo Alto, Calif.) to detect chlamydial elementary bodies in smears and an enzyme immunoassay (EIA) (Chlamydiazyme; Abbott Laboratories, North Chicago, Ill.). The performance of these tests in different evaluations has varied widely, perhaps because of the different levels of expertise of the evaluators in performing either the test being evaluated or the reference TC method. The type of population tested also makes a considerable difference. The tests are not as accurate for a low-risk group as for a high-risk group. The reported sensitivity of DFA testing has ranged from 60 to 99% (4, 9, 19), and that of EIA has ranged from 60 to 98% (2, 7, 18). What has become clear is that EIA and DFA testing are useful in high-prevalence settings, in which 2 to 3% false-positive rates still allow a high predictive value for a positive result. These tests are also useful when culturing is not available or when transportation of TC specimens is a problem.

It is also clear that the performance of the nonculture tests can be improved. One target for improvement is specimen quality. Optimum collection of epithelial cells is a major factor in the sensitivity of most chlamydia tests. Low sensitivity can be the result of poor sample collection. The DFA test offers the advantage of determining whether an adequate sample has been obtained. In our laboratory, approximately 10% of cervical fluorescent-antibody smears from some clinics have an insufficient number of cells. A proposed solution to this problem has been the introduction of the endocervical cytobrush. Three published studies found the cytobrush to yield an increase in the number of epithelial cells, chlamydial elementary bodies (EBs), and positivity rate in direct immunofluorescence (3, 8; R. B. Broughton, Jr., S. Cox, K. Forrest, J. Findlay, and B. Judson, Program Abstr. 27th Intersci, Conf. Antimicrob. Agents Chemother., abstr. no. 724, 1987). That experience has not been universal (21), indicating a need for further evaluation.

In this study we determined whether the cytobrush was a better collection device for obtaining endocervical specimens for TC isolation and DFA testing.

MATERIALS AND METHODS

Patient population. Specimens were collected from 632 nonpregnant females attending a sexually transmitted disease clinic. The patients screened were under 30 years old and were often symptomatic.

Specimen collection. The study was divided into two sections, with all sampling being randomized. In the first half, TC (Calgiswab type I), DFA testing (cytobrush and Dacron swab type I), and EIA (Dacron swab type I) were performed on 303 patients. The swabs for DFA testing and EIA were the standard ones supplied in the kits. For the remaining 329 patients, the cytobrush was used for collecting specimens for TC, and the other specimens were collected as described above. Briefly, the exocervix was cleaned of excess mucus and exudate. The swab or cytobrush was inserted into the endocervical canal, rotated against the wall, and removed, avoiding contact with any vaginal surface.

For TC, the specimen was placed into transport medium (2.0 ml of Eagle minimal essential medium in Earle salts containing 10% fetal calf serum, 1% L-glutamine [200 nM solution], 10 μg of gentamicin per ml, 100 μg of vancomycin per ml, 10 U of nystatin [Mycostatin; E. R. Squibb & Sons, Princeton, N.J.] per ml, and 0.003 mmol of glucose per ml). Specimens were refrigerated for up to 72 h until inoculated into cells. For DFA testing, we covered the 8-mm well of the MicroTrak slide evenly with the sample. The smear was air dried, fixed by flooding with methanol, and air dried again.

* Corresponding author.

TABLE 1. Comparison of cytobrush versus swab samples for detection of *C. trachomatis*

Test and result	No. of specimens[a]		Test and result	No. of specimens[a]	
	TP	TN		TP	TN
Isolation with Calgiswab (n = 267)			Isolation with cytobrush (n = 301)		
Positive	20	0	Positive	37	0
Negative	9	238	Negative	0	264
MicroTrak with Dacron swab (n = 568)			Microtrak with cytobrush (n = 568)		
Positive	45	5	Positive	56	12
Negative	21	497	Negative	10	490
Chlamydiazyme with Dacron swab (n = 568)					
Positive	56	10			
Negative	10	492			

[a] TP, True-positives, i.e., specimens that were TC (isolation) positive and/or both MicroTrak and Chlamydiazyme positive; TN, true-negatives.

The Dacron swab for Chlamydiazyme was placed into the EIA transport tube (containing a storage reagent and a biostatic substance to retard growth of the organisms). For all test comparisons, each procedure was performed blindly.

MicroTrak procedure. Smears were overlaid with 30 μl of the fluorescent-antibody reagent. After incubation for 15 min at room temperature in a moist chamber, slides were rinsed in distilled H_2O and air dried. One drop of MicroTrak mounting fluid and a cover slip were applied to each smear. We used a Zeiss epifluorescence microscope with a quartz halogen light source (12 V, 100 W) and a fluorescein isothiocyanate filter system. All specimens were screened at 400×, and every field was scanned. Positives were confirmed at 1,000×, having 10 or more smooth, evenly fluorescing, bright apple green disks consistent with chlamydial EBs. Each slide was graded with the number of EBs seen (up to 100). Negative slides had fewer than 10 EBs. Inadequate smears contained mostly mucus and few or no epithelial cells. Thick smears had layers of cells (squamous cells, epithelial cells, erythrocytes, or polymorphonuclear cells), blood, and mucus that made screening of the slides impossible.

Chlamydiazyme procedure. One milliliter of specimen dilution buffer was added to each sample and incubated at room temperature for 15 min. Tubes were vortexed for three cycles of 15 s each. Excess fluid was removed from the swab, and the swab was discarded. Specimens (200 μl each) and controls were placed in reaction tray wells. The first incubation (37°C for 1 h) contained treated beads. The second incubation contained rabbit antibody to *C. trachomatis*. The third incubation contained horseradish peroxidase-conjugated antibody to rabbit immunoglobulin G. For color development, the beads were transferred to tubes and incubated at room temperature with 300 μl of *o*-phenylenediamine substrate for 30 min. One milliliter of 1 N sulfuric acid was added to stop the reaction, and a Quantum II spectrophotometer was used to read all the samples at A_{492}.

TC procedure. Using a modification of the procedure of Ripa and Mardh (12), we inoculated samples onto McCoy cells within 72 h of collection. The 1-dram (ca. 3.7-ml) shell vials were centrifuged (2,700 × *g*) for 1 h, and the monolayers were treated with cycloheximide. After 48 h of incubation at 35°C under 5% CO_2, one vial of each specimen was fixed with ethanol and stained with fluorescent antibody (Syva culture confirmation reagent). Cover slips were scanned at 250× for typical apple green inclusions of *C. trachomatis*. The number of inclusions seen per cover slip was scored. All specimens were blindly passaged at 4 days and treated in the same manner.

Statistical methods. Sensitivity, specificity, and predictive values were calculated by standard techniques (5). Statistical comparisons were performed with the Yates corrected x^2 analysis, the McNemar test for changes, and a paired t test.

RESULTS

A total of 632 specimens were collected. However, only 568 specimens were used for direct comparisons. The other 64 specimens were excluded because of unmatched pairs, inadequate smears, and TC specimens that were too old to process or had cytopathic effects (CPE). For analysis, true-positives were identified as TC positive and/or both DFA and EIA positive.

The overall prevalence of infection was 11.6%. The positivity rates for the two parts of the study were similar at 10.9% (29 of 267) and 12.3% (37 of 301). However, isolation rates for TC swab and cytobrush were 7.5 and 12.3%, respectively ($P < 0.05$). In the first part of the study, we observed a TC (swab) sensitivity of only 69% and a 10% CPE rate. Of the 20 TC (swab) positives, 14 (70%) were identified in the first passage, with an average of 40 inclusions per 12-mm cover slip. When cytobrushes were used to collect specimens in the latter half of the study, TC (brush) sensitivity increased to 100% ($P < 0.001$), with only 2.7% CPE. Of the 37 TC (brush) positives, 30 (81%) were isolated in the first passage, with an average of 275 inclusions per 12-mm cover slip. The increases in the number of specimens positive at first passage and the mean inclusion counts were statistically significant ($P < 0.001$ and $P < 0.02$, respectively). Tables 1 and 2 show the comparisons of swabs versus cytobrushes in TC and DFA testing.

Of the 632 DFA (swab) and DFA (brush) smears collected, 4.5 and 1.0% were inadequate, respectively ($P < 0.001$). Only one DFA (brush) specimen was unreadable, owing to a

TABLE 2. Performance profiles of diagnostic tests for *C. trachomatis* with cytobrushes and swabs for collection[a]

Test	Sensitivity (%)	Specificity (%)	Predictive value (%)	
			Positive	Negative
TC (Calgiswab type I)	69	100	100	96
TC (cytobrush)	100	100	100	100
DFA (Dacron swab type I)	68	99	90	96
DFA (cytobrush)	85	98	82	98
EIA (Dacron swab type I)	85	98	85	98

[a] The prevalence of true positives was 11.6% (66 of 568).

TABLE 3. Effect of various swabs and the cytobrush on the growth of two *C. trachomatis* isolates

Time (h) and strain	Mean no. of inclusions/3- to 12-mm cover slip with:				
	No swab (control)	Cytobrush	Calgiswab (type I)	Dacron swab (type I)	Rayon swab (type I)
0					
1	220	218	174	262	230
2	331	468	215	292	201
24					
1	72	83	81	98	33
2	231	156	80	67	75
48					
1	61	59	45	51	62
2	123	197	48	80	82
72					
1	12	17	31	26	36
2	84	65	41	44	59

thick smear. We found the sensitivity of DFA (swab) versus DFA (brush) testing to be 68 versus 85% ($P < 0.001$). Specificity was equivalent between DFA (brush) and DFA (swab) testing (98 and 99%, respectively). The DFA (brush) system detected an additional 11 true-positive smears that the DFA (swab) system missed. Thus, positivity went from 8.8% (swab) to 12% (cytobrush). Of the 68 smears positive in DFA (brush) testing, 48 (71%) contained ≥100 EBs. With DFA (swab) testing, only 25 of 50 (50%) positive smears contained ≥100 EBs ($P < 0.05$). The difference between the number of EBs seen in DFA (swab) versus DFA (brush) testing was significant ($P < 0.001$).

Analyzing our MicroTrak data with a positive cutoff value of two EBs would generate 3 more true-positive and 10 false-positive results for DFA (brush) testing. One true-positive and two false-positive results would be added for DFA (swab) testing. Thus, sensitivity and specificity would change to 89 and 96% with DFA (brush) and 70 and 99% with DFA (swab) testing, respectively.

As an adjunctive observation, the effect of the swabs and the cytobrush was tested in vitro against two freshly isolated strains of chlamydiae. Table 3 shows the viability of the organisms over a 72-h period.

DISCUSSION

This study clearly demonstrates that the cytobrush is superior to the commonly used swabs for the collection of cervical chlamydia specimens. Tables 1 and 2 show that the cytobrush outperformed the swabs in both the TC and DFA detection methods.

When we compared cytobrush versus swab for MicroTrak, there was a marked increase in the number of evaluable smears, EB counts, and positivity rate. The sensitivity of the test increased with the brush, while the specificity was essentially equivalent. Judson and Lambert (6) also reported an improved performance, as did Broughton et al. (27th ICAAC), who found a statistically significant increase in the number of positive smears with the brush.

Other endpoints have been used to determine DFA positivity (19). Changing our cutoff to two EBs would slightly increase the sensitivity but would also lower the specificity with both swab and brush. We therefore recommend the standard cutoff of 10 EBs or more for a positive MicroTrak test. With the methanol (rather than acetone) fixation step, the performance of the MicroTrak has greatly improved (16). With the cytobrush, the sensitivity has increased from 68 to 85%. In a previous study (4) at this same clinic (prevalence of chlamydia, 20%), we observed a 76% sensitivity with the swab and acetone fixation.

It is known that culturing is less than 100% sensitive (14). This is a constant problem in nonculture test evaluations. Our previous estimates of TC sensitivity were on the order of 75 to 85%, although others have felt the test was better in their experiments (13, 15). At 69%, the rather low sensitivity of TC (swab) was a concern. However, in addition to this poor performance, 10% of our cultures had CPE. In most cases, CPE usually denote the collection of excess mucus and exudate. When TC (brush) was used, our sensitivity increased to 100%, with only 2.7% CPE (a more acceptable rate). The average number of inclusions per 12-mm cover slip seen on first passage also increased with brush use. The cytobrush likely increased the positivity rate and the number of organisms isolated because of better sampling of columnar epithelial cells. Collection of multiple swabs can also increase chlamydia isolation (11).

In a recent report Weiland et al. (21) found no difference between the Dacron swab and the cytobrush in the DFA test. It was concluded that both devices were comparable for the detection of *C. trachomatis*. However, they did not state whether inadequate smears were obtained by either method. This number would reflect proper specimen collection by the physicians and/or nurses. If the clinicians are particularly adept at collecting specimens with a swab, then a cytobrush might not make a difference. The same would probably hold true for culturing. Our results suggest that the key manageable variable in TC results is variation in collected specimens. Laboratories having a better TC sensitivity than our reported 75 to 85% probably had better-trained specimen collectors. With the better specimens obtained with the brush, we have seen, for the first time, a TC sensitivity of 100%. Studies of cytobrush use on asymptomatic patients are now needed to test DFA suitability for screening a low-risk population.

The EIA performed as well as the DFA test (Tables 1 and 2). We did not evaluate the cytobrush in the EIA because this test largely detects soluble antigens and is less likely to be as dependent on epithelial cell sampling as are the DFA test and culturing.

Because different swabs are known to affect the growth of chlamydiae (10), we tested both cytobrushes and swabs in vitro against two strains of *C. trachomatis* (Table 3). The Calgiswabs we use are prescreened to avoid toxic swabs. We found that the cytobrush produced roughly the same viability patterns as the Dacron, rayon, and calginate swabs. Therefore, the brush was equally toxic for the chlamydiae and the McCoy cells.

In summary, we believe that the cytobrush is the preferred device for collecting cervical chlamydia specimens from nonpregnant women. However, there are some disadvantages to the cytobrush. Firstly, the brush has not been evaluated on pregnant women; therefore, it cannot yet be recommended for use on this population. Secondly, clinicians may have to be retrained in the collection method. If applied vigorously, the brush may cause bleeding and thus discomfort, especially in women displaying a friable cervix. Clinicians do not need to rub as hard to collect an adequate specimen with the brush as they do with the swab, so less bleeding would be seen as collectors gain more experience with the use of the brush. In our study bleeding was not a

problem. Finally, the cytobrush ($0.33) is more expensive than the Calgiswab ($0.15). We feel the added cost is well justified by the increase in test sensitivity.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grant AI 21912 from the National Institutes of Health and by Syva Co.

LITERATURE CITED

1. **Centers for Disease Control.** 1985. *Chlamydia trachomatis* infections. Policy guidelines for prevention and control. Morbid. Mortal. Weekly Rep. **34**:53s–74s.
2. **Chernesky, M. A., J. B. Mahoney, S. Castriciano, M. Mores, I. O. Stewart, S. J. Landis, W. Seidelman, E. J. Sargeant, and C. Leman.** 1986. Detection of *Chlamydia trachomatis* antigens by enzyme immunoassay and immunofluorescence in genital specimens from symptomatic and asymptomatic men and women. J. Infect. Dis. **154**:141–148.
3. **Ciotti, R. A., S. J. Sondheimer, and I. Nachamkin.** 1988. Detecting *Chlamydia trachomatis* by direct immunofluorescence using a cytobrush sampling technique. Genitourin. Med. **64**:245–246.
4. **Forbes, B. A., N. Bartholoma, J. McMillan, M. Roefaro, L. Weiner, and L. Welych.** 1986. Evaluation of a monoclonal antibody test to detect *Chlamydia* in cervical and urethral specimens. J. Clin. Microbiol. **23**:1136–1137.
5. **Griner, P. F., R. J. Mayewski, A. I. Mushlin, and P. Greenland.** 1981. Selection and interpretation of diagnostic tests and procedures: principles and applications. Ann. Intern. Med. **94**:553–600.
6. **Judson, B. A., and P. P. Lambert.** 1988. Improved Syva MicroTrak *Chlamydia trachomatis* direct test method. J. Clin. Microbiol. **26**:2657–2658.
7. **Lefebvre, J., H. Laperriere, H. Rousseau, and R. Masse.** 1988. Comparison of three techniques for detection of *Chlamydia trachomatis* in endocervical specimens from asymptomatic women. J. Clin. Microbiol. **26**:726–731.
8. **Linder, L. E., J. A. Nettum, S. L. Miller, and K. H. Altman.** 1987. Comparison of scrape, swab and cytobrush samples for the diagnosis of cervical chlamydial infection by immunofluorescence. Diagn. Microbiol. Infect. Dis. **8**:179–182.
9. **Lipkin, E. S., J. V. Moncada, M. A. Shafer, T. E. Wilson, and J. Schachter.** 1986. Comparison of monoclonal antibody staining and culture in diagnosing cervical chlamydial infection. J. Clin. Microbiol. **23**:114–117.
10. **Mardh, P.-A., and B. Zeeberg.** 1981. Toxic effect of sampling swabs and transportation test tubes on the formation of intracytoplasmic inclusions of *Chlamydia trachomatis* in McCoy cell cultures. Br. J. Vener. Dis. **57**:268–272.
11. **Paavonen, J.** 1979. *Chlamydia trachomatis*-induced urethritis in female partners of men with nongonococcal urethritis. Sex. Transm. Dis. **6**:69–71.
12. **Ripa, K. T., and P.-A. Mardh.** 1977. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. J. Clin. Microbiol. **6**:328–331.
13. **Schachter, J.** 1985. Immunodiagnosis of sexually transmitted disease. Yale J. Biol. Med. **58**:443–452.
14. **Schachter, J.** 1986. Rapid diagnosis of sexually transmitted diseases—speed has a price. Diagn. Microbiol. Infect. Dis. **4**:185–189.
15. **Schachter, J., and D. H. Martin.** 1987. Failure of multiple passages to increase chlamydial recovery. J. Clin. Microbiol. **25**:1851–1853.
16. **Schachter, J., J. Moncada, C. R. Dawson, J. Sheppard, P. Courtright, M. E. Said, S. Zaki, S. F. Hafez, and A. Lorincz.** 1988. Nonculture methods for diagnosing chlamydial infection in patients with trachoma: a clue to the pathogenesis of the disease? J. Infect. Dis. **6**:1347–1352.
17. **Schachter, J., E. Stoner, and J. Moncada.** 1983. Screening for chlamydial infections in women attending family planning clinics—evaluation of presumptive indicators for therapy. West. J. Med. **138**:375–379.
18. **Smith, J. W., R. E. Rogers, B. P. Katz, J. F. Brickler, P. L. Lineback, B. Van Der Pol, and R. B. Jones.** 1987. Diagnosis of chlamydial infection in women attending antenatal and gynecologic clinics. J. Clin. Microbiol. **25**:868–872.
19. **Thomas, B. J., R. T. Evans, D. A. Hawkins, and D. Taylor-Robinson.** 1984. Sensitivity of detecting *Chlamydia trachomatis* elementary bodies in smears by use of fluorescein labeled monoclonal antibody: comparison with conventional chlamydial isolation. J. Clin. Pathol. **37**:812–816.
20. **Washington, A. E., R. E. Johnson, and L. L. Sanders.** 1987. *Chlamydia trachomatis* infections in the United States: what are they costing us? J. Am. Med. Assoc. **257**:2070–2072.
21. **Weiland, T. L., K. L. Noller, T. F. Smith, and S. J. Ory.** 1988. Comparison of Dacron-tipped applicator and cytobrush for detection of chlamydial infections. J. Clin. Microbiol. **26**:2437–2438.