JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1996, p. 534–536 Vol. 34, No. 3
0095-1137/96/$04.00+0

Is Urine Leukocyte Esterase Test a Useful Screening Method To Predict *Chlamydia trachomatis* Infection in Women?

JOAN M. CHOW,[1] JEANNE MONCADA,[1] DAVID BROOKS,[1] GAIL BOLAN,[2] HOWARD SHAW,[2] AND JULIUS SCHACHTER[1]*

University of California, San Francisco,[1] and San Francisco City Clinic,[2] San Francisco, California

Received 29 September 1995/Returned for modification 6 November 1995/Accepted 18 December 1995

We evaluated the use of the leukocyte esterase test (LET) on first-catch urine specimens from women as a screening test to predict infection with *Chlamydia trachomatis*. For diagnosis, we used Abbott's ligase chain reaction (LCR) on urine specimens and isolation by tissue culture (TC) on cervical brushes. Of 4,053 women attending sexually transmitted disease and family planning clinics, 4.3% ($n = 174$) were positive by TC and 5.9% ($n = 239$) were positive by LCR. When LET was compared to TC, the sensitivity, specificity, positive predictive value, and negative predictive value were 54.0, 67.0, 6.8, and 97.0%, respectively. The corresponding performance of LET versus LCR was 53.1, 67.3, 10.1, and 95.8%. Almost half of the laboratory-confirmed chlamydial infections were negative by LET. The low specificity probably reflects multiple causes of pyuria in women and results in a low positive predictive value. LET is neither sensitive nor specific as a predictor of chlamydial infection and cannot be recommended for use as a screening test for *C. trachomatis* with first-catch urine samples from females from low- or moderate-prevalence populations.

Chlamydia trachomatis is the most prevalent sexually transmitted bacterial disease in the United States, with an annual incidence of greater than four million infections (4). Consequences of these infections in women include pelvic inflammatory disease, tubal infertility, and ectopic pregnancy (3, 7). An effective control strategy would include screening and treatment. In the past, the most sensitive method for identification of chlamydial infections has been tissue culture (TC) isolation of cervical and urethral swab specimens. This method, even with optimal sampling and transport conditions, is a time-consuming and labor-intensive process not readily adapted for screening of large populations. Subsequent development of antigen detection assays, such as enzyme immunoassays (EIAs), has increased the availability of screening and the convenience of specimen handling. EIAs are now being used with first-catch urine (FCU) from symptomatic males to detect *C. trachomatis* (12). However, there are numerous drawbacks to their use: they are recommended primarily for screening of symptomatic men and are less sensitive than TC. EIAs have performed poorly in detecting chlamydial infections in female FCU. There continues to be a need for a rapid, sensitive, specific, and noninvasive diagnostic assay for chlamydial infections. Prescreening or selective screening with such an assay would enrich the yield of infections and result in a greater potential for cost-effective chlamydia screening.

The leukocyte esterase test (LET) detects the presence of an esterase that is released by polymorphonuclear leukocytes in urine. It is an inexpensive, rapid, and convenient alternative to microscopic examination for polymorphonuclear leukocytes in the clinic setting. Past studies of the LET using FCU from asymptomatic males reported various sensitivities in the range of 41 to 100% and specificities of 52 to 100% (10, 12, 13, 18, 19). Shafer and colleagues were able to demonstrate that a screening strategy based upon prescreening of male FCU with LET followed by a confirmed EIA (positives retested by direct fluorescent-antibody staining) had a useful performance profile (67% sensitivity and 100% specificity) and was cost-effective (18, 19). On the basis of results from male studies, the LET has been considered for screening of female FCU as well. The performance of the LET on women has been compared to TC isolation of *C. trachomatis* and culture of *Neisseria gonorrhoeae* from cervical swabs by Lewis and colleagues (9). They reported the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of LET to be 81, 67, 60, and 85%, respectively.

Ligase chain reaction (LCR; Abbott Laboratories, Abbott Park, Ill.) is a DNA detection method for *C. trachomatis* infections which provides amplification of target sequences within the chlamydial cryptic plasmid. In one large multicenter evaluation, LCR was able to detect 94.4% of cervical infections versus 65% detection by TC (17). With female FCU, LCR also has a comparable sensitivity of 87.5 to 96.3% versus 55.6 to 67.1% by TC (2, 5, 8, 16). LCR has also been reported to be as sensitive (93.5 to 96.4%) for male FCU (5, 6). Given the significant increase in sensitivity offered by LCR, we reevaluated LET with female FCU by using LCR and TC as the "gold standard."

MATERIALS AND METHODS

Specimen collection. Female patients attending the San Francisco City Clinic Sexually Transmitted Disease Clinic and the San Francisco General Hospital Teen Family Planning Clinic were screened for *C. trachomatis*. The majority of these patients were asymptomatic. Initially, 25 to 50 ml of FCU was obtained in a sterile collection cup. A pelvic examination was then performed. Excess mucus or discharge was removed from the cervix with a large cotton swab or sponge. A cytobrush was rotated against the endocervical canal and placed into chlamydia 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 mycostatin per ml, and 0.003 mmol of glucose per ml). All specimens were stored at 4°C until transport to the laboratory.

TC isolation. For TC isolation, we used a modification of the procedure of Ripa and Mardh (14). Specimens were inoculated onto McCoy cells in 1-dram (3.697-ml) shell vials within 72 h of collection. The specimens were centrifuged (2,700 × *g*) for 1 h, and monolayers were treated with cycloheximide (1 μg/ml). After 48 h of incubation at 35°C under 5% CO_2, one vial of each specimen was fixed with ethanol and stained with a fluorescent antibody (Syva MicroTrak *C. trachomatis* Culture Confirmation Reagent). Coverslips were scanned at a mag-

* Corresponding author. Mailing address: Chlamydia Research Laboratory, Building 30, Room 416, San Francisco General Hospital, 1001 Potrero Avenue, San Francisco, CA 94110. Phone: (415) 824-5115. Fax: (415) 821-8945.

TABLE 1. Performance profile of LET[a] with female FCU against cervical TC and LCR for detection of *C. trachomatis*

Result	TC or LCR		
	Positive[b]	Negative	Total
LET positive	139	1,236	1,375
LET negative	131	2,547	2,678
Total	270	3,783	4,053

[a] Prevalence, 6.7%; sensitivity, 51.5%; specificity, 67.3%; PPV, 10.1%; NPV, 95.1%.
[b] Confirmed positive by TC and/or LCR.

nification of ×250 for typical apple green inclusions of *C. trachomatis*. The number of inclusions seen per coverslip was scored. All negative specimens (positives were all amplified by passage and stored) were blind passaged at 4 days and treated in the same manner.

LET. For LET, urine specimens were stored at 4°C for up to 72 h after collection. Most of the specimens were tested within 24 h; approximately 10 to 15% were tested at 72 h (specimens collected on Friday were tested on Monday). Before testing, the samples were brought to room temperature and mixed thoroughly. The LET dipstick (Chemstrip 2 LN; Boehringer Mannheim, Indianapolis, Ind.) was dipped into urine (approximately 1 s) and read within 1 to 2 min. The test result was rated according to a Boehringer Mannheim four-item color scale (zero, trace, 1+, 2+). A result of trace or above was considered positive.

To ensure that LET results were not affected by a maximum 72-h postcollection interval, we tested 89 urine specimens within 24 and 72 h postcollection. Five (5.6%) had a modest change in LET result (three from trace to negative, one from 2+ to 1+, and one from negative to trace).

LCR assay. The LCR test has been previously described (8). The assay targets DNA sequences within the *C. trachomatis* cryptic plasmid. Urine specimens were stored at 4°C and batch tested with 7 days of collection; all samples were treated through the heating stage within 4 days. For sample preparation, 1 ml of FCU was placed into a microcentrifuge tube and cytospun ($13{,}000 \times g$) for 10 min. The supernatant was aspirated. The urine pellet was then resuspended in LCR buffer and heated at 100°C for 15 min. For DNA amplification, 100 ml of each sample and control was added to a microcentrifuge tube containing a predispensed LCR mixture of four oligonucleotide probes and a thermostable enzyme (ligase). The tube was inserted into a Perkin Elmer 280 Thermocycler programmed for 40 cycles of 1 s at 97°C, 1 s at 55°C, and 50 s at 62°C. An automated microparticle EIA was used to detect amplicons on Abbott's IMX. Total assay time was 4 to 5 h per 36 samples.

Resolution of discrepant LCR results. Samples that were TC negative and LCR positive were further evaluated by direct fluorescent-antibody assay. The remnants of the original TC specimen were microcentrifuged for 20 min. Smears were made from the sediment, air dried, and fixed with methanol. Syva MicroTrak *C. trachomatis* direct fluorescent antibody was used to stain the slide. Detection of ≥2 elementary bodies was considered a positive result. If the direct fluorescent-antibody assay was negative, the FCU was tested by LCR with probes targeting a portion of the *C. trachomatis* major outer membrane protein gene. If neither the major outer membrane protein gene nor elementary bodies were detected, the original positive LCR result was considered a false positive.

Statistical methods. Sensitivity, specificity, and predictive values were calculated by standard techniques (15). Statistical comparisons were made by using the z statistic for comparing proportions.

RESULTS

Of the 4,053 women tested, 4.3% ($n = 174$) were positive for chlamydial infection by TC versus 5.9% ($n = 239$) by LCR. When LET was tested against the expanded gold standard (TC- and/or LCR-confirmed positive result), the sensitivity, specificity, PPV, and NPV were 51.5, 67.3, 10.1, and 95.1%, respectively (Table 1).

Table 2 shows the performance profile of LET compared with those of the TC and LCR standards separately. The LET sensitivity and the specificity were approximately the same for both standards. The PPV increased from 6.8% with TC to 10.1% with the more sensitive LCR standard. The NVP decreased from 97.0 to 95.8%.

Table 3 shows the distribution of first-pass positive TC specimens by inclusion count (≤100 and >100 inclusions) and LET result. The difference in the percentages of samples negative by LET between the low- and high-titer groups was of borderline statistical significance: 52.2% LET negative in the ≤100-inclusion group versus 36.1% LET negative in the >100-inclusion group ($P = 0.0574$).

TABLE 2. LET performance as measured against TC and LCR standards[a]

Standard result	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
TC positive	54.0	67.0	6.8	97.0
LCR positive	53.1	67.3	10.1	95.8

[a] A total of 4,053 samples were tested.

DISCUSSION

In this study, LET performance as measured against TC and LCR was clearly not adequate for a recommendation that it be used as a screening tool for chlamydial infections in females. The sensitivity and specificity were poor: almost half of the infections were in women with a negative LET. Previous performance profiles of the LET with male and female FCU were based upon a comparison with TC (1, 9, 10, 13, 18, 19). It was thought that a more sensitive diagnostic test might improve LET performance. With the use of LCR on FCU, 65 more infections were detected than with TC of cervical specimens. However, rather than detection of more chlamydial infections in LET-positive FCU specimens, it was found that many of the LCR-positive specimens were LET negative. Consequently, LET performance is particularly poor in light of this expanded comparison.

Previous work done by Lewis and colleagues (9) evaluated the performance of LET in detecting both gonococcal and chlamydial infections. Their sample ($n = 206$) had a high gonococcus prevalence (35%) and a moderate *C. trachomatis* prevalence (10%). Only 22 TC-positive chlamydial specimens were tested against LET, and no data were offered for LET performance specifically with respect to chlamydial infections. Thus, our study data are not directly comparable to those of Lewis' study. The gonococcus prevalence in this population was roughly 2.5% (4.0% sexually transmitted disease clinic, 0.9% family planning clinic), and the chlamydial prevalence was 7%. We do not have matched gonococcus results for this particular study, but the low prevalence of gonococci in our population would not alter the conclusion that LET does not perform well in low-prevalence populations. A positive LET could result from chlamydial or gonococcal infection, and any screening approach would have a higher PPV in a higher-prevalence setting. Obviously, there are reasons for a positive LET other than chlamydial or gonococcal infections, and in the low-prevalence populations, these compromise LET as a screening tool for these bacterial sexually transmitted diseases.

We found that positive LET results were correlated with

TABLE 3. Analysis of specimens positive for *C. trachomatis* by TC with regard to inclusion count and LET result[a]

LET result	No. (%) of samples with:		*P* value
	≤100 inclusions	>100 inclusions	
0	59 (52.2)	22 (36.1)	0.0574
Trace	12 (10.6)	6 (9.8)	
1+	19 (16.8)	11 (18.0)	
2+	23 (20.3)	22 (36.1)	
Total	113 (100.0)	61 (100.0)	

[a] A total of 4,053 samples were tested.

infectious burdens, as higher-titer chlamydial specimens were more likely to be LET positive. This finding may explain the lower sensitivity of LET for detection of chlamydial infections in asymptomatic populations. Asymptomatic chlamydial infections are more likely to be associated with lower infectious burdens, and our study population was predominantly asymptomatic.

The low sensitivity of the LET for chlamydiae may be due to its dependency upon the amount of inflammation in the urethra. If the main site of infection is the cervix, there may not be a concurrent chlamydial infection in the urethra. In fact, in a subset of women (n = 2,812) for whom urethral cultures were performed in this study (17), only 34% of cervical TC-positive women (49 of 142) had concurrent urethral infections. Further, of these women with urethral infections, only 52% were LET positive. It is possible that low LET sensitivity is due to low-titer urethral infections, but this subgroup of our results was too small to confirm this (of 50 positive urethral specimens, 26 were LET positive and 24 were LET negative; 24 of the LET-positive samples had <100 inclusions, and 22 of the LET-negative samples had <100 inclusions).

Ultimately, it would be desirable to enrich yields of infections detected in screening and thereby make *C. trachomatis* diagnostic testing more cost-effective. However, it is clear with these study results that the use of LET on female FCU will not have that effect, particularly in low-prevalence populations. On the basis of the low LET sensitivity and specificity with female FCU in these populations, we cannot recommend the LET for routine screening of chlamydial infections in women.

ACKNOWLEDGMENT

This work was supported by National Institutes of Health grant AI31499.

REFERENCES

1. **Adger, H., M. A. Shafer, R. L. Sweet, and J. Schachter.** 1984. Screening for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in adolescent males: value of the first catch urine examination. Lancet **ii:**944–945.
2. **Bassiri, M., H. Y. Hu, M. A. Domeika, J. Burczak, L.-O. Svensson, H. H. Lee, and P.-A. Mardh.** 1995. Detection of *Chlamydia trachomatis* in urine specimens from women by ligase chain reaction. J. Clin. Microbiol. **33:**898–900.
3. **Cates, W., Jr., and J. R. Wasserheit.** 1991. Genital chlamydial infections: epidemiology and reproductive sequelae. Am. J. Obstet. Gynecol. **164:**1771–1781.
4. **Centers for Disease Control.** 1993. Recommendations for the prevention and management of *Chlamydia trachomatis* infections. Morbid. Mortal. Weekly Rep. **42:**1–39.
5. **Chernesky, M. A., D. Jang, H. Lee, J. D. Burczak, H. Hu, J. Sellors, S. J. Tomazic-Allen, and J. B. Mahony.** 1994. Diagnosis of *Chlamydia trachomatis* infections in men and women by testing first-void urine by ligase chain reaction. J. Clin. Microbiol. **32:**2682–2685.
6. **Chernesky, M. A., H. H. Lee, J. Schachter, J. D. Burczak, W. E. Stamm, W. M. McCormack, and T. C. Quinn.** 1994. Diagnosis of *Chlamydia trachomatis* urethral infection in symptomatic and asymptomatic men by testing first-void urine in a ligase chain reaction assay. J. Infect. Dis. **170:**1308–1311.
7. **Chow, J., M. L. Yonekura, G. A. Richwald, S. Greenland, R. L. Sweet, and J. Schachter.** 1990. The association between *Chlamydia trachomatis* and ectopic pregnancy. JAMA **263:**3164–3167.
8. **Lee, H. H., M. A. Chernesky, J. Schachter, J. D. Burczak, W. W. Andrews, S. Muldoon, G. Leckie, and W. E. Stamm.** 1995. Diagnosis of *Chlamydia trachomatis* genitourinary infection in women by ligase chain reaction assay of urine. Lancet **345**(8944)**:**213–216.
9. **Lewis, J., S. Kraus, and J. Lossick.** 1994. Use of the urine leukocyte esterase test to screen women for gonorrhea and chlamydia infections, abstr. C-350, p. 552. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
10. **McNagny, S. E., R. M. Parker, J. M. Zenilman, and J. S. Lewis.** 1992. Urinary leukocyte esterase test: a screening method for the detection of asymptomatic chlamydial and gonococcal infections in men. J. Infect. Dis. **165:**573–576.
11. **Moncada, J., H. H. Lee, D. Brooks, K. Ray, and J. Schachter.** 1995. Use of cervical and first catch urine to identify *Chlamydia trachomatis* in women, abstr. C-486, p. 85. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
12. **Moncada, J., J. Schachter, M. A. Shafer, E. Williams, L. Gourlay, B. Lavin, and G. Bolan.** 1994. Detection of *Chlamydia trachomatis* in first catch urine samples from symptomatic and asymptomatic males. Sex. Transm. Dis. **21:** 8–12.
13. **Patrick, D. M., M. L. Rekart, and L. Knowles.** 1994. Unsatisfactory performance of the leukocyte esterase test of first voided urine for rapid diagnosis of urethritis. Genitourin. Med. **70:**187–190.
14. **Ripa, K. T., and P.-A. Mårdh.** 1977. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. J. Clin. Microbiol. **6:**328–331.
15. **Schachter, J.** 1985. Immunodiagnosis of sexually transmitted disease. Yale J. Biol. Med. **58:**443–452.
16. **Schachter, J., J. Moncada, R. Whidden, H. Shaw, G. Bolan, J. Burczak, and H. Lee.** 1995. Noninvasive tests for diagnosis of *Chlamydia trachomatis* infection: application of ligase chain reaction to first catch urine specimens of women. J. Infect. Dis. **172:**1411–1414.
17. **Schachter, J., W. E. Stamm, T. C. Quinn, W. W. Andrews, J. Burczak, H. H. Lee.** 1994. Ligase chain reaction to detect *Chlamydia trachomatis* infection of the cervix. J. Clin. Microbiol. **32:**2540–2543.
18. **Shafer, M. A., J. Schachter, J. Moncada, J. Keogh, R. Pantell, L. Gourlay, S. Eyre, and C. Boyer.** 1993. Evaluation of urine-based screening strategies to detect *Chlamydia trachomatis* among sexually active asymptomatic young males. JAMA **1270:**2065–2070.
19. **Shafer, M. A., J. Schachter, A. B. Moscicki, A. Weiss, J. Shalwitz, E. Vaughan, and S. G. Millstein.** 1989. Urinary leukocyte esterase screening test for asymptomatic chlamydial and gonococcal infections in males. JAMA **262:**2562–2566.