

Evaluation of Laboratory Testing Methods for *Chlamydia trachomatis* Infection in the Era of Nucleic Acid Amplification

TAMARA J. BATTLE,^{1,4} MATTHEW R. GOLDEN,^{1,3*} KATHLEEN L. SUCHLAND,¹ JON M. COUNTS,²
JAMES P. HUGHES,⁵ WALTER E. STAMM,¹ AND KING K. HOLMES¹

Division of Infectious Diseases, Center for AIDS & STD,¹ and Department of Biostatistics,⁵ University of Washington, Public Health–Seattle & King County,³ and Washington State Public Health Laboratory,² Seattle, Washington, and College of Medicine, Howard University, Washington, D.C.⁴

Received 9 April 2001/Returned for modification 4 May 2001/Accepted 26 May 2001

Diagnostic tests presently available for *Chlamydia trachomatis* have widely varying performance characteristics. To assess evolving laboratory testing practices since the introduction of nucleic acid amplification tests (NAAT), we surveyed laboratories in Washington State about their testing practices in 1998 and compared our findings to a similar survey conducted in 1995. Laboratory directors of 61 (87%) of 70 laboratories performing chlamydial tests in 1998 returned a survey. Between 1995 and 1998, 36 laboratories discontinued chlamydial testing, and the total number of laboratories performing tests in the state decreased from 92 to 70, a 24% decline. Of the 36 laboratories that discontinued testing, 25 (69%) had previously used rapid tests. While no laboratory routinely used NAAT in 1995, ligase chain reaction (LCR) was used in 23% of laboratories in 1998 and accounted for 113,624 (36%) of the 318,133 tests performed that year. Among the remaining 204,509 tests performed in 1998, other tests employed included DNA probe (29%), enzyme immunoassay (20%), culture (12%), direct fluorescent antibody assays (3%), and rapid tests (<1%). The majority (65%) of tests performed in 1998 using technologies other than LCR or culture were done in laboratories that did more than 10,000 tests. Cost and loss of revenue to laboratories were the most frequently cited reasons for not adopting NAAT. We conclude that in Washington State, NAAT have been rapidly adopted in larger laboratories, but most patients are still tested with much less sensitive technologies. Financial constraints represent the major barrier to more widespread use of DNA amplification tests.

Screening programs and diagnostic testing for *Chlamydia trachomatis* infection are now in place in much of the United States (4). The sensitivities of available laboratory tests for *C. trachomatis* vary from less than 40 to almost 100% (1, 9, 10, 11). However, national guidelines for chlamydial testing have not been revised since the introduction of nucleic acid amplification tests (NAAT) (2), and relatively little has been published about what tests are used in actual practice.

In 1995, a survey of laboratories testing for *C. trachomatis* in Washington State found that 43% employed low-sensitivity rapid tests (12). Although the U.S. Food and Drug Administration approved the first NAAT in 1993, no laboratory in Washington State reported using such tests routinely in 1995. Since that time, NAAT have become widely available. To assess current laboratory testing practices and determine how they have changed since the introduction of NAAT, we surveyed laboratories in Washington State regarding their testing practices in 1998 and compared our findings to those of the 1995 survey.

MATERIALS AND METHODS

Surveys were conducted in 1995 and in 1999. The 1999 survey asked directors about their laboratory's testing practices in 1998. For both surveys, study investigators attempted to contact all laboratories in Washington State registered with the State Department of Health Office of STD Services or the Washington State Department of Health Office of Quality Assurance to perform tests for *C. trachomatis* in the preceding year. In addition, as part of the survey regarding

testing practices in 1998, directors of laboratories included in the 1995 survey that were no longer identified by the state as performing chlamydial tests in 1998 were contacted by telephone and, if their laboratories were still performing chlamydial testing, added to the study population.

Procedures for the 1995 survey have been described previously (12). The survey on testing practices in 1998 was a one-page, 10-question instrument that was sent to each laboratory's director to assess the laboratory's diagnostic testing practices for *C. trachomatis*. Directors were asked to provide information about their laboratory's size and affiliation (public health, commercial, hospital-affiliated, university-based, clinic/doctor office), what testing technologies they routinely employed (ligase chain reaction [LCR], PCR, Gen-Probe, culture, direct fluorescent antibody, enzyme immunoassay [EIA], rapid test), and the numbers of tests performed and cases detected using each test in 1998. (The 1995 survey asked for number of tests performed within a range of categories and did not collect data about the number of positive tests.) Directors were also asked to provide their laboratory's standard charge for testing; no effort was made to determine how much payers actually reimbursed laboratories. In addition, if laboratories were not using NAAT, laboratory directors were asked open-ended and multiple-choice questions to identify barriers to adopting such technology.

If laboratory directors did not respond within 2 weeks of the original mailing, a second survey was sent to them. Thereafter, directors were contacted by telephone to follow up on surveys not returned and to obtain missing information for incomplete surveys.

To assess how testing practices had changed since the introduction of NAAT, results from the survey on testing in 1998 were compared to those obtained in the 1995 survey. Results were summarized with percentages for binary data and medians for continuous data. Fisher's exact test was used to compare the frequency of use of different types of tests between 1995 and 1998. All analyses were performed using the SPSS and SAS programs.

RESULTS

Seventy-five laboratories were registered with the Washington State Department of Health Office of STD Services and the Washington State Department of Health Office of Quality

* Corresponding author. Mailing address: Harborview Medical Center, Box 359931, 325 9th Ave, Seattle, WA 98104-2499. Phone: (206) 731-6829. Fax: (206) 731-4151. E-mail: golden@u.washington.edu.

TABLE 1. Characteristics of laboratories performing diagnostic tests for *C. trachomatis* in Washington State, 1998

Parameter	No. (%) of laboratories	
	1994 (n = 89)	1998 (n = 61)
Type of laboratory ^a		
Hospital-affiliated	30 (38)	28 (46)
Clinic or physician's office	31 (39)	12 (20)
Private commercial	14 (18)	7 (12)
Government	4 (5)	5 (8)
University	1 (1)	1 (2)
No. of tests performed annually		
<120	10 (11)	9 (15)
120–599	32 (36)	16 (26)
600–1,199	17 (19)	8 (13)
1200–5,999	20 (22)	15 (25)
≥6,000	10 (11)	13 (21)
Most frequently used testing technology ^b		
LCR	0	14 (23)
EIA	22 (25)	18 (30)
Direct fluorescent antibody	18 (20)	7 (12)
Gen-Probe	7 (8)	11 (18)
Culture	4 (4)	0
Rapid test	38 (43)	11 (18)

^a No data available for nine laboratories surveyed in 1995.

^b *P* < 0.001 comparing 1994 and 1998.

Assurance to perform chlamydial testing in 1998. Directors of 68 (91%) of these laboratories returned a survey, 8 of whom reported that their laboratories no longer performed tests for *C. trachomatis*. Directors of an additional 36 laboratories included in the 1995 survey but no longer identified by the state as performing tests for *C. trachomatis* were contacted by telephone. Of these, 33 (89%) confirmed that their laboratories no longer performed tests to detect *C. trachomatis* and 3 revealed that they were still performing such tests. Of these three, one returned a survey. Thus, 61 (87%) of 70 laboratories believed to be testing for *C. trachomatis* in Washington State provided information about their testing practices.

Types of laboratories, numbers of tests performed, and primary technologies employed in 1995 and in 1998. Between the two survey periods, the number of laboratories performing tests for *C. trachomatis* decreased from 92 to 70, a 24% decline.

The survey indicated that this decline was attributable largely to a drop in the number of clinic- or office-based laboratories performing small numbers of tests, especially rapid tests (Table 1). Of the 89 laboratories surveyed in 1995, 36 (40%) had ceased to perform *Chlamydia* tests by 1998. Rapid tests were used by 25 (69%) of the laboratories that discontinued testing, and 66% of all laboratories performing rapid tests in 1995 ceased to do so over a 3-year period. Between the two survey periods, 25 laboratories were newly registered to perform chlamydial tests in Washington State. Of these, 21 (84%) returned surveys, of which 16 reported actually performing tests for *C. trachomatis* in 1998: five (31%) used LCR, five (31%) used rapid tests, three (19%) used EIA, one (6%) used Gen-Probe, and two (12%) used direct fluorescent antibody assays.

In 1998, 36% of all tests performed in laboratories participating in the study were done using LCR, and another 12% were done using culture (Table 2). The remaining 52% of tests were performed using less-sensitive technologies, including DNA probe (26%), EIA (17%), direct fluorescent antibody assays (2%), and rapid in-office tests (0.6%). Only a single laboratory reported using LCR to confirm positive tests done by EIA. Of note, five large laboratories performed 115,638 (56%) of the 204,509 tests done with technologies other than LCR or culture. While the median charges for culture and LCR were higher than for other tests, the charges reported for different tests overlapped considerably across laboratories.

Rationale for persistent use of low-sensitivity tests. The directors of laboratories using technologies other than LCR were asked multiple-choice and open-ended questions about why their laboratories did not use an NAAT or send specimens to another laboratory for such testing. While the cost of NAAT was the most frequently cited reason (Table 3), 36 (65%) of 55 directors of laboratories not performing NAAT responded that limitations of their laboratories—test complexity, space requirements, or test volume—were barriers preventing them from adopting such a test. The need for laboratories to maintain revenue was the most frequently cited reason for not sending specimens to other laboratories for testing (Table 4). Relatively few laboratory directors (19%) cited a belief that other chlamydial tests were as good as NAAT as a reason for not sending specimens to other laboratories.

TABLE 2. Testing volume by technology type

Technique	No. (%) of labs performing test ^a	Median no. of tests performed (range)	No. of tests performed (% of all tests performed)	No. of positive tests (% of total no. of positive tests)	% Positive (range)	Median charge per test (US\$) (range)
LCR	16 (23)	4,320 (265–42,322)	113,624 (35.7)	5,198 (41.9)	4.6 (1.4–8.4)	42 (7.9–62) ^b
DNA probe	10 (13)	6,205 (40–30,334)	91,909 (28.9)	3,203 (25.8)	3.5 (2.4–7.5)	35.50 (6–46) ^c
EIA	19 (28)	1,820 (29–25,736)	63,841 (20.1)	2,084 (16.8)	3.3 (0.8–4.7)	32.19 (15.50–64.6) ^d
Culture	5 (8)	773 (178–34,393)	37,502 (11.8)	1,541 (12.4)	4.1 (2.3–5)	67.55 (38.90–104) ^e
Direct fluorescent antibody	12 (13)	184 (15–4,661)	9,377 (2.9)	297 (2.4)	3.2 (0–8.6)	36 (21–86) ^f
Rapid test	11 (15)	160 (6–489)	1,880 (0.6)	72 (0.6)	3.8 (0–6.9)	37 (19–45)
Total	60 (100)	1,139 (6–53,045)	318,133	12,395	4.0 (0–8.6)	38.0 (7.9–104)

^a Some laboratories performed more than one type of test.

^b No charge was defined for 42,322 tests performed in a public health laboratory.

^c No charge was defined for 7,089 tests performed in private or hospital laboratories and 6,205 tests performed in a military laboratory.

^d No charges were defined for 25,736 tests performed in a hospital lab and 22,486 tests performed in other labs.

^e No charge was defined for 773 tests performed in a lab.

^f No charge was defined for 143 tests performed in labs.

TABLE 3. Reasons cited by laboratory directors for not adopting NAAT to detect *C. trachomatis*^a

Reason	No. (%) of laboratories
Cost.....	31 (56)
Inadequate testing volume.....	23 (42)
Current test sufficiently sensitive for population.....	19 (36)
Space limitations.....	16 (29)
Clientele satisfied with current test.....	15 (27)
Test complexity.....	13 (24)
Lack of confidence in DNA testing.....	1 (2)

^a Based on responses from 55 laboratory directors of labs not performing nucleic acid amplification testing.

DISCUSSION

We conducted serial surveys of laboratories in Washington State in order to assess changes in testing practices for *C. trachomatis* between 1995 and 1998. At the time of the second survey, 36 (40%) of the laboratories that participated in the 1995 survey had discontinued testing, while 18 new laboratories began testing. Concurrent with this contraction in the number of laboratories performing tests, LCR testing was adopted, primarily by large laboratories. In 1998, approximately one-third of all tests performed in the state were done using LCR. No laboratories reported routinely using Amplicor PCR or amplified Gen-Probe, a finding that was confirmed in discussions with Roche Diagnostics and Gen-Probe representatives in the state. In contrast, the use of rapid tests, the least sensitive of the available technologies, declined dramatically. Most of the small laboratories that used rapid tests in 1995 ceased to perform any chlamydial tests by 1998, and rapid tests were used for less than 1% of tests in the state in 1998.

Despite the adoption of NAAT in many large laboratories, most patients were still tested with less sensitive tests in 1998. Because the reported sensitivities of the different tests have varied widely in published reports, we cannot precisely estimate the number of false-negative tests in the state attributable to the use of less-sensitive tests. However, assuming that NAAT are 90% sensitive, that, relative to NAAT, culture is 50 to 90% sensitive (11), and that EIA, direct fluorescent antibody, and Gen-Probe are 45 to 65% sensitive (9), between 3,217 and 8,454 cases of *C. trachomatis* infection, or 21 to 40% of all cases, may have been missed as a result of using tests other than NAAT. Although NAAT are more expensive, cost-effectiveness data support their use either alone (6) or as confirmatory tests for specimens with reactive EIAs in the negative "gray zone" (3).

TABLE 4. Reasons cited by laboratory directors for not sending specimens to other laboratories for DNA amplification testing^a

Reason	No. (%) of laboratories
Loss of revenue.....	21 (45)
Cost of test.....	16 (34)
Prohibitive turnaround time.....	14 (30)
Belief that DNA amplification is not superior.....	9 (19)
Option to send test out unavailable.....	3 (6)

^a Based on responses from 47 laboratory directors of labs not performing or sending specimens to other labs for DNA amplification testing.

Financial barriers was the most frequently cited reason for a laboratory's not adopting more sensitive NAAT. This constraint may diminish with more widespread use of specimen pooling (7, 8), as well as confirmatory NAAT testing of specimens with results in the negative gray zone on EIA (3), and as competition among NAAT lowers costs and price. However, in the absence of revised national guidelines favoring NAAT, the ever-increasing emphasis on cost containment may inhibit wider adoption of newer and more expensive tests. Moreover, for most small-volume laboratories, limited laboratory space and expertise and potential financial losses will remain barriers to adopting these more complex tests. These laboratories could be encouraged to refer specimens to larger laboratories offering NAAT. In any case, small-volume laboratories perform relatively few tests in Washington State; 65% of all lower-sensitivity tests (EIA, direct fluorescent antibody, DNA probe, or rapid tests) were performed in laboratories doing more than 10,000 tests per year, and only 22% of such tests were done in laboratories performing fewer than 5,000 tests per year. The consolidation of testing into a small numbers of large laboratories may provide an opportunity for public health officials to significantly increase the yield of chlamydial screening programs through efforts to persuade laboratory directors to change testing technologies.

In conclusion, we have documented the rapid adoption of NAAT for *C. trachomatis* in Washington State between 1995 and 1998 and the phasing out of rapid tests used in smaller laboratories. Increasingly, testing is being concentrated in larger laboratories. However, many of these laboratories continue to use lower-sensitivity and less-costly tests. Cost remains a formidable barrier to the more widespread adoption of NAAT, and there may be instances in which presently available rapid tests, despite their low sensitivity, are more cost-effective than NAAT. For example, point-of-care rapid tests may ensure proper treatment of persons considered unlikely to return for test results (5). However, given the superior sensitivity of NAAT, the ability to extend screening to community-based settings with these tests using urine and self-collected vaginal swabs and data supporting the relative cost-effectiveness of this technology for detecting chlamydial infection, more widespread adoption of these tests is warranted. Public health officials should actively promote the use of NAAT.

ACKNOWLEDGMENTS

T.J.B. was supported by NIH training grant T35 AI 07616. M.R.G. was supported by NIH postdoctoral training grant NIAID AI07149 and by University of Washington NIH STD Cooperative Research Center AI31448.

REFERENCES

1. Black, C. M. 1997. Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. Clin. Microbiol. Rev. 10:160-184.
2. Centers for Disease Control and Prevention. Recommendations for the prevention and management of *Chlamydia trachomatis* infections, 1993. Morb. Mortal. Wkly. Rep. Morb. Mortal. Wkly. Rep. 42(RR-12):1-39.
3. Dean, D., D. Ferrero, and M. McCarthy. 1998. Comparison of performance and cost-effectiveness of direct fluorescent-antibody, ligase chain reaction, and PCR assays for verification of chlamydial enzyme immunoassay results for populations with a low to moderate prevalence of *Chlamydia trachomatis* infection. J. Clin. Microbiol. 36:94-99.
4. Division of STD Prevention. 2000. Sexually transmitted disease surveillance, 1999. Department of Health and Human Services. Centers for Disease Control and Prevention, Atlanta, Ga.
5. Gift, T. L., M. S. Pate, E. W. Hook 3rd, and W. J. Kassler. 1999. The rapid

- test paradox: when fewer cases detected lead to more cases treated: a decision analysis of tests for *Chlamydia trachomatis*. *Sex. Transm. Dis.* **26**:232–240.
6. **Howell, M. R., T. C. Quinn, W. Brathwaite, and C. A. Gaydos.** 1998. Screening women for *Chlamydia trachomatis* in family planning clinics: the cost-effectiveness of DNA amplification assays. *Sex. Transm. Dis.* **25**:108–117.
 7. **Kacena, K. A., S. B. Quinn, M. R. Howell, G. E. Madico, T. C. Quinn, and C. A. Gaydos.** 1998. Pooling urine samples for ligase chain reaction screening for genital *Chlamydia trachomatis* infection in asymptomatic women. *J. Clin. Microbiol.* **36**:481–485.
 8. **Kapala, J., D. Copes, A. Sproston, J. Patel, D. Jang, A. Petrich, J. Mahony, K. Biers, and M. Chernesky.** 2000. Pooling cervical swabs and testing by ligase chain reaction are accurate and cost-saving strategies for diagnosis of *Chlamydia trachomatis*. *J. Clin. Microbiol.* **38**:2480–2483.
 9. **Marrazzo, J. M., and W. E. Stamm.** 1998. New approaches to the diagnosis, treatment, and prevention of chlamydial infection. *Curr. Clin. Top. Infect. Dis.* **18**:37–59.
 10. **Newhall, W. J., R. E. Johnson, S. DeLisle, D. Fine, A. Hadgu, B. Matsuda, D. Osmond, J. Campbell, and W. E. Stamm.** 1999. Head-to-head evaluation of five *Chlamydia* tests relative to a quality-assured culture standard. *J. Clin. Microbiol.* **37**:681–685.
 11. **Schachter, J., W. E. Stamm, T. C. Quinn, W. W. Andrews, J. D. Burczak, and H. H. Lee.** 1994. Ligase chain reaction to detect *Chlamydia trachomatis* infection of the cervix. *J. Clin. Microbiol.* **32**:2540–2543.
 12. **Suchland, K. L., J. M. Counts, and W. E. Stamm.** 1997. Laboratory methods for detection of *Chlamydia trachomatis*: survey of laboratories in Washington State. *J. Clin. Microbiol.* **35**:3210–3214.