Laboratory Methods for Detection of *Chlamydia trachomatis*: Survey of Laboratories in Washington State

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The last decade has witnessed the development of a wide variety of diagnostic tests for *Chlamydia trachomatis*. In order to determine what laboratory methods are being used to detect *C. trachomatis* infections in Washington State and to identify factors influencing test selection, between April 1995 and October 1995 we conducted a mailed questionnaire survey of all 112 laboratories certified to do chlamydia testing. Of these, 20 had discontinued testing for *C. trachomatis*, and responses were obtained from 89 (97%) of the remaining 92 laboratories. Surprisingly, 38 (43%) of the 89 laboratories used rapid tests such as Clearview and Surecell, making such tests the most commonly used laboratory tests. Laboratories which used rapid tests had lower test volumes, less experience performing tests for *C. trachomatis*, less frequent attendance at professional meetings, and greater reliance on manufacturers for information compared with laboratories which used other methods. Confirmation of non-culture-positive results was provided by 28 (34%) of the 82 laboratories doing non-culture-based tests. Forty-one (47%) of 88 laboratories reported having compared their method with another method. Test volume was the strongest predictor of laboratories which confirmed positive non-culture-based test results and which had performed a laboratory comparison of methods. We conclude that rapid tests for *C. trachomatis* are often being used inappropriately and that efforts are needed to improve effective implementation and quality assurance of laboratory testing for *C. trachomatis*.

Chlamydia trachomatis is the most common bacterial sexually transmitted disease in the United States. More than 4 million infections occur annually, costing an estimated \$2.4 billion (15, 16). These costs are largely attributable to the sequelae of chlamydial infections in women, especially pelvic inflammatory disease (PID), infertility, and ectopic pregnancy. In Washington State, 9,462 cases of *C. trachomatis* infection were reported in 1995, with the ratio of the number of reported cases in women exceeding those in men by 4:1 as a result of screening practices that target women (17).

Women with untreated chlamydial infection are at increased risk for PID (11), infertility (13), and ectopic pregnancy (14). Untreated infections also serve as a reservoir for the continued transmission of disease. The majority of infections in women are asymptomatic and thus require laboratory testing for diagnosis (2, 9). As a result, laboratory screening of young women has been considered a cornerstone of chlamydia control programs (4). Chlamydia screening programs have been associated both with subsequent reductions in the prevalence of chlamydia in a given population (1, 3) and with a reduced incidence of PID among the women screened (11).

Over the past decade, the use of laboratory tests for the detection of *C. trachomatis* has increased dramatically due both to the development of new testing methods and to the heightened awareness of the prevalence of asymptomatic infections and the importance of screening programs. The wide variety of tests on the market and the rate at which new and improved technologies are being developed make it difficult to choose the best test. As stated in a recent editorial, "this large number of tests in so many different formats actually creates a problem for the ultimate end-users in deciding which tests are

best suited for their specific needs" (10). To help improve laboratory practices in this complex environment, the Centers for Disease Control and Prevention (CDC) has also developed and distributed guidelines for laboratories performing diagnostic tests for chlamydia tests (4).

In order to determine what laboratory methods were being used in Washington State and to ascertain whether these laboratories were following CDC recommendations, we conducted a mailed questionnaire survey of all laboratories certified to do chlamydia testing in Washington State. The data gathered described the characteristics of laboratories that may affect their choice of testing method and their compliance with CDC guidelines.

MATERIALS AND METHODS

Sampling procedure. The population surveyed consisted of all laboratories that performed tests for *C. trachomatis* in the state of Washington. There were 112 laboratories on lists provided by the Washington State Office of STD Services and the Washington State Department of Health Office of Quality Assurance. Of these, 20 laboratories reported that they no longer tested for chlamydia, 77 responded by mail, and 12 were interviewed over the telephone.

Survey questionnaire. An 8-page questionnaire consisting of 35 questions was designed to collect information describing (i) the test method being used in the laboratory, (ii) factors important in test selection, (iii) performance of procedures recommended by CDC, (iv) the respondent's beliefs about test reliability, (v) and demographic information about the laboratory. A cover letter describing the purpose of the study was directed to the laboratory supervisor or person responsible for selecting the test method used to detect chlamydia. The questionnaire was mailed three times between April and October 1995, and telephone interviews were conducted with the 12 laboratories that did not respond by mail. Telephone interviews consisted of 13 questions from the questionnaire which were considered to be most important.

Operational definition of the variables. The dependent variables were (i) test method used by the laboratory to detect *C. trachomatis*, (ii) performance of a confirmatory test for samples originally found to be positive, and (iii) performance of an assessment comparing the method in use with an appropriate standard.

Test methods were categorized as culture, enzyme immunoassay (EIA), DNA or RNA probe assay, direct immunofluorescence (direct fluorescence assay [DFA]), chemiluminometric immunoassay, PCR, leukocyte esterase assay, rapid in-office test and serological assay. If more than one test was performed in the

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same laboratory, the method used most often was considered the test method for statistical analysis. In some cases, the brand name of the test was used to clarify method category. Rapid in-office tests included Clearview, Surecell, Testpack, and QuickVue.

The performance of a confirmatory test was defined by whether a laboratory performed a confirmatory test on all, some, or none of the samples initially found to be positive for chlamydia. CDC recommends that laboratories verify non-culture-positive test results when a false-positive test result is likely to have adverse medical, social, or psychological consequences. CDC further recommends that positive test results be confirmed by culture, by a second but different non-culture-based test, or by use of a blocking antibody or competitive probe.

The performance of an in-house assessment of laboratory methods was defined by asking whether the laboratory had ever compared the sensitivity and specificity of two or more test methods for chlamydia by performing a comparison by using each method with the same or a duplicate sample. The CDC recommendation actually states that each laboratory should verify the accuracy of non-culture-based test methods by periodically comparing non-culture-based test results with those of a high-quality culture system.

The independent variables were factors that may have influenced the selection of the test method, the confirmation of positive results, or the laboratory comparison of methods. These included test-related factors such as sensitivity and specificity and laboratory-related factors such as experience, sources of information, level of education of staff, test volume, available resources, accessibility to other laboratories, and beliefs about test reliability.

Data analysis. SPSS software was used to analyze the data. Initially, the chi-square test of association was used to examine univariate relationships between variables. Logistic regression was applied to the analysis of those variables found to be significantly associated with the dependent variables (namely, rapid in-office test use, performance of confirmatory tests, and performance of a laboratory comparison of methods) in order to adjust for the confounding effect of test volume.

RESULTS

Response rate. Of 112 questionnaires sent, 77 were returned by mail and 12 were completed over the telephone; 20 laboratories had discontinued testing for chlamydia. Thus, the response rate of those laboratories doing testing was 97% (89 of 92).

Demographic characteristics of the laboratories. Most laboratories (64 of 89; 72%) were located in counties with greater than 100,000 inhabitants. The highest density of laboratories occurred in counties with populations exceeding 1 million. Rural counties with populations of less than 10,000 inhabitants comprised 7% (6 of 89) of the laboratories, with 19 of the laboratories (21%) located in counties with populations of between 10,000 and 100,000 people. The majority of laboratories (66%) processed fewer than 100 tests for chlamydia each month (Table 1). Almost half (49%) were within 5 miles of another laboratory that also conducted tests for chlamydia. A fairly high percentage (33%) had 3 years or less of experience with testing for chlamydia. In more than half of the laboratories (55%), the test method was selected by a person with a bachelor's degree in medical technology or microbiology. The minimum educational requirement for a person performing tests for chlamydia was less than a bachelor's degree in most laboratories (56%).

Laboratory methods used to detect *C. trachomatis*. Surprisingly, a rapid in-office test was the method most frequently used to identify chlamydia (43%), followed by EIA (25%) and DFA (20%) (Table 2). Many laboratories performed testing by more than one method (19%), but for statistical analysis the method most often used for the initial identification of chlamydia was considered the primary method. The DFA method was often used as a secondary or confirmatory test. Culture was performed in 12 laboratories, but only 4 routinely used it for initial testing. The DNA probe was offered by 10 laboratories and was used for initial testing in 7 laboratories. Only one laboratory in Washington State reported performing serology for chlamydia (Table 2).

Confirmation of positive results and laboratory comparison. Most laboratories that used non-culture-based test methods

TABLE 1. Demographic characteristics of the laboratories

Characteristic (units or measure)	Measurement	Number of laboratories (% of total) ^a
Test volume (no. of chlamydia tests performed monthly)	0-9	10 (11)
	10-49	32 (36)
	50-99	17 (19)
	100-499	20 (22)
	500 or more	10 (11)
		89 (total)
Location (no. of mi from nearest laboratory that also performs tests for chlamydia)	Less than 5	38 (49)
	5-19	24 (31)
	20 or more	15 (19)
		77 (total)
Experience (no. of yr of testing for chlamydia)	1-3	29 (33)
	4-6	24 (28)
	7-10	20 (23)
	More than 10	14 (16)
		87 (total)
Education		
Level of education of person selecting test method	High school	2 (2)
	Associate degree	6 (7)
	Bachelor's degree	48 (55)
	Master's degree	8 (9)
	M.D.	14 (16)
	Ph.D.	9 (10)
		87 (total)
Field of education of person selecting test method	Lab medicine	34 (41)
	Microbiology	20 (24)
	Medicine	14 (17)
	Other	15 (18)
		83 (total)
Minimum level of education of person performing the test	None	3 (4)
	High school	15 (19)
	Associate degree	25 (32)
	Bachelor's degree	34 (44)
		77 (total)

^a The total number of responses was less than 89 in some cases because not all questions were answered by all respondents.

reported that they do not confirm positive results (66%). Thirteen laboratories reported that they confirm some of their positive results, and 15 reported that they confirm all tests for chlamydia that were initially found to be positive.

Less than half of the laboratories (47%) reported having performed a laboratory comparison of methods before implementing a test for chlamydia.

Characteristics of laboratories using rapid in-office test methods. Most laboratories that used rapid in-office test methods (25 of 38; 66%) were located in counties with populations of greater than 100,000 inhabitants. Twenty-four (63%) rapid test users were classified as physician office laboratories, 6 (16%) were clinics, 5 (13%) were hospitals, and 2 (5%) were independent laboratories; 1 laboratory designated itself as "other."

In general, laboratories that used rapid in-office test methods performed fewer than 100 tests for chlamydia each month, had fewer than 3 years of experience with testing for chlamydia, and were unfamiliar with the PCR method (Table 3).

TABLE 2. Laboratory methods used for identification of *C. trachomatis* in Washington State laboratories

Method $\pm$ (brand)	No. (%) of laboratories using main method of identification	No. (%) laboratories in which the test is available
Rapid in-office test	38 (43)	38 (43)
Clearview (Wampole)	18	
Surecell (Kodak)	16	
Testpack (Abbott)	2	
QuickVue (Quidel)	2	
EIA	22 (25)	24 (27)
Microtrak (Syva)	8	
Chlamydiazyme (Abbott)	7	
Vidas (Biomérieux)	6	
Ortho (Meridian)	1	
DFA	18 (20)	28 (31)
Microtrak (Syva)	15	
Vidas (Biomérieux)	1	
Bartels	1	
Pathodx (Diagnostic Products)	1	
DNA or RNA probe (Genprobe)	7 (8)	10 (11)
	7	
Culture	4 (4)	12 (13)
Ortho (Meridian)	1	
Bartels	1	
In house	1	
Not specified	1	
PCR	0	2 (2)
Serology	0	1 (1)
Total	89	

Many of the selectors of rapid tests had a bachelor's degree in medical technology or microbiology. Technical simplicity was considered a primary requirement for test selection. Rapid tests were often performed by personnel without bachelor's degrees. They reported that they obtained their training in performing the test procedure by reading the package insert. Users of rapid tests obtained their information from manufacturers and sales representatives more often than users of other methods. They also attended fewer meetings and relied less often on papers published in professional journals for their information. They were more likely to believe that manufacturers' claims about test reliability were reasonably accurate and rarely believed that tests do not perform as claimed by the manufacturer. Rapid test users were more likely than users of other methods to perceive a lack of available resources.

Logistic regression analysis revealed that test volume did not account for the association between the use of a rapid method and the experience or education of the test performer, the requirement that the test be simple, and beliefs about the reliability of claims by manufacturers. It was also noted that laboratories that used a rapid in-office method were likely to be located relatively close to another laboratory that also performed tests for chlamydia.

Characteristics of laboratories with low test volumes. Test volume was highly associated with test method, confirmation of

positive results, and performance of laboratory comparisons of methods. Table 4 describes the characteristics of laboratories that were directly related to test volume. Most laboratories with low test volumes used either a DFA or a rapid in-office test method, did not confirm their positive results, and rarely performed a comparison of test methods. Laboratories with low test volumes had less experience than laboratories with medium or high volumes with testing for chlamydia, and they were less familiar with PCR. They also perceived their available resources to be relatively inadequate. The most commonly reported missing resource was information, followed by money and time (data not shown). Personnel of laboratories with low test volumes attended meetings less often, relied on information from manufacturers and sales representatives more often, and published papers less often than laboratories with higher test volumes. In addition, the minimum educational requirement in laboratories with low test volumes was likely to be less than the requirement in larger laboratories, and training in performing the test procedure was more likely to have been obtained by reading the package insert in laboratories with low test volumes. Neither the location of the laboratory nor the educational level of the test selector appeared to be associated with test volume.

DISCUSSION

Our survey presents a quantitative description of many of the characteristics of laboratories that perform diagnostic tests for *C. trachomatis* in Washington State. Although a descriptive study cannot determine causal relationships, several of the associations that we observed have interesting and disturbing implications. Most laboratories that performed tests for *C. trachomatis* were small, processing fewer than 100 tests for chlamydia each month, and small laboratory size was associated with the use of a DFA or rapid in-office test, nonperformance of confirmatory tests for positive results, and nonperformance of a laboratory comparison of methods. Because they are likely to obtain their information from test manufacturers and sales representatives, laboratory technicians in small laboratory settings may be unaware of the limitations of their tests, the importance of the confirmation of positive results in certain circumstances, and the need to perform periodic comparisons of the method being used with an external standard. Many small laboratories reported that they have inadequate resources to achieve such goals and may be unable to afford more accurate tests, more complete quality assurance activities, or comparative testing. Since many of these laboratories were within 5 miles of another laboratory that performs tests for chlamydia, centralization of testing might be one possible solution. This might also facilitate comparative testing. Small laboratories need to be advised of the limitations of their methods and the importance of recommendations for quality assurance activities. If they lack the resources to perform recommended procedures, they should be encouraged to send their chlamydia specimens to a laboratory with a test volume that supports more complete quality assurance.

Of considerable importance was the unexpected finding that the most commonly used method was a rapid in-office test. Although most of the laboratories that use these tests processed fewer than 100 tests each month, we estimate that 16,920 rapid tests were performed in Washington State in 1994 (data extrapolated from the survey). Studies performed in experienced laboratories have indicated that these rapid tests are relatively insensitive compared with other methods (6, 8). Furthermore, rapid in-office tests are intended for use only with high-risk populations, in which a rapid test result can be used

TABLE 3. Characteristics of laboratories using rapid in-office methods

Characteristic	No. positive/total no. (%)		<i>P</i> value ^a	Adjusted <i>P</i> -value ^b
	Rapid test users	Other test users		
Test volume: less than 100 tests/mo	37/38 (97)	22/51 (43)	0.000	NA
Experience: less than 3 yr of testing for chlamydia	23/36 (64)	6/51 (12)	0.000	0.000
Awareness: familiarity with PCR	15/37 (41)	38/51 (75)	0.001	0.101
Education: bachelor's degree or above	30/38 (79)	50/51 (98)	0.004	0.774
Education: associate degree or less	13/30 (43)	5/47 (11)	0.001	0.009
Test requirement: simplicity	22/36 (61)	14/51 (27)	0.002	0.006
Source of training: package insert	26/38 (68)	16/51 (31)	0.001	0.095
Belief: most tests do not perform as claimed	3/37 (8)	21/51 (41)	0.002	0.011
Belief: manufacturer claims are reasonably accurate	22/29 (76)	22/47 (47)	0.013	0.009
Resources: perceived to be adequate	18/28 (64)	40/47 (85)	0.037	0.385
Source of information: ever attended a meeting	13/29 (45)	35/47 (74)	0.009	0.267
Source of information (for test sensitivity): manufacturer	21/28 (75)	23/46 (50)	0.034	0.886
Source of information (for test selection): published papers	18/34 (53)	38/50 (76)	0.028	0.301
Source of information (for test selection): sales representatives	11/34 (32)	7/50 (14)	0.044	0.815
Location: <5 mi from nearest laboratory performing chlamydia tests	15/30 (50)	24/47 (51)	0.927	0.028
Confirmatory test: confirm none of positive results	27/37 (73)	28/48 (58)	0.161	0.698
Laboratory comparison: performed comparison of test methods	13/37 (35)	28/51 (55)	0.066	0.619

^a Chi-square test of association.^b Logistic regression adjusted for the number of tests performed monthly. NA, not applicable.

to treat the patient immediately and thus reduce the number of infected women who are lost to follow-up between diagnosis and treatment. The use of rapid in-office tests in low-risk and pregnant patients or in situations when the rapid test result will not be used to immediately direct therapy is inadvisable and represents poor medical practice. More sensitive, laboratory-based testing should be done in these situations.

In most laboratories, including laboratories with low test volumes and those that use rapid tests, the person selecting the test method for chlamydia had at least a bachelor's degree in the field of microbiology or medical technology. This suggests that the curriculum in clinical microbiology courses needs to reemphasize the importance of thoroughly evaluating new tests before implementing them in the laboratory. The apparent lack of association between educational level and compliance with CDC recommendations may also imply that factors other than education largely determine laboratory practice. The data suggested that many physicians chose rapid in-office

tests. In fact, half of the laboratories in which the test selector was a physician (8 of 16 laboratories) used a rapid in-office test (data not shown). Further study is needed to determine whether physicians are aware of the lack of sensitivity of these methods and to assess their motivation for selecting these tests. Small laboratories and laboratories that used rapid methods obtained more information from manufacturers and sales representatives than was common in larger laboratories, in which meeting attendance and journal reading were more common. This suggests that in order to reach the small laboratories, it will be necessary to develop an outreach method of disseminating information similar to that used by manufacturers.

Since 1993, when the CDC recommendations were published, nothing related to assessments of their degree of implementation has been reported. Our data suggest that less than half of the laboratories in Washington State follow the CDC recommendations to confirm the results for selected samples with positive non-culture-based test results and to

TABLE 4. Characteristics of laboratories with low volumes^a

Characteristic	No. positive/total no. (%)		<i>P</i> value ^b
	Laboratories with low vol	Laboratories with medium or high vol	
Test method: use of DFA or rapid method	51/59 (86)	5/30 (17)	0.000
Confirmation: confirm none of positives	44/57 (77)	11/28 (39)	0.001
Comparison: performed laboratory comparison	19/58 (33)	22/30 (73)	0.000
Experience: 3 yr or less	26/57 (46)	3/30 (10)	0.001
Awareness: familiarity with PCR	29/58 (50)	24/30 (80)	0.006
Resources: perceive to be adequate	31/46 (67)	27/29 (93)	0.010
Source of information: ever attended a meeting about chlamydia	24/47 (51)	24/29 (82)	0.005
Source of information (for test sensitivity): manufacturer	33/46 (71)	11/28 (39)	0.006
Source of information (for test selection): published papers	31/55 (56)	25/29 (86)	0.006
Source of information (for test selection): sales representatives	17/55 (31)	1/29 (3)	0.003
Source of training: package insert	33/59 (56)	9/30 (30)	0.020
Education: Bachelor's degree or above	51/59 (86)	29/30 (97)	0.130
Education: Associate degree or less	15/48 (31)	3/29 (10)	0.036
Location: <5 mi from nearest laboratory performing chlamydia tests	23/48 (48)	16/29 (55)	0.537

^a Low volume was considered to be <100 chlamydia tests/month.^b Chi-square test of association.

perform a laboratory comparison of methods before implementing testing for chlamydia. Recommended procedures, including the performance of laboratory comparisons and the confirmation of positive results, increase the sensitivities and specificities of tests for chlamydia (5, 7, 12) and should be common practice in chlamydia laboratories. Although following the CDC recommendations presumably increases the accuracy and value of laboratory testing for chlamydia, it is not required by law. There is a need to identify factors that will motivate laboratories to implement the CDC recommendations effectively.

The information collected in this survey provided a quantitative description of laboratory practices related to testing for chlamydia in Washington State. The high response rate (97%) lends validity to the fact that the data are representative for laboratories in Washington State. It is not known whether the survey findings can be extrapolated to laboratories outside Washington State. Since there has been both considerable research related to chlamydia testing and to the development of chlamydia screening programs in Washington State, testing may well be more widespread in Washington State than elsewhere. Variations in state regulation of laboratories, manufacturers' marketing strategies, the reimbursement policies of insurance companies, and access to reference laboratory facilities would all affect the laboratory practices described in this report and likely vary from state to state. A larger study with a broad geographic base would be needed in order to eliminate the regional bias inherent in this study. Such a study would also be of interest to confirm and provide a better understanding of the reasons behind the widespread use of rapid in-office tests, to define the current degree of implementation of diagnostic tests such as PCR and ligase chain reaction that have become commercially available since our survey, and to better understand the limited performance of the laboratory procedures recommended by CDC.

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