JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1986, p. 1029–1033
0095-1137/86/121029-05$02.00/0

Vol. 24, No. 6

Effect of Blind Passage and Multiple Sampling on Recovery of *Chlamydia trachomatis* from Urogenital Specimens

ROBERT B. JONES,[1,2]* BARRY P. KATZ,[1,3] BARBARA VAN DER POL,[1] VIRGINIA A. CAINE,[1,4] BYRON E. BATTEIGER,[1] AND WILBERT J. NEWHALL V[1]

Departments of Medicine[1] and Microbiology and Immunology,[2] Indiana University School of Medicine, Indianapolis, Indiana 46223; Regenstrief Institute for Health Care Research, Indianapolis, Indiana 46202[3]; and Marion County Health Department, Indianapolis, Indiana 46204[4]

Received 9 June 1986/Accepted 18 August 1986

Detection of chlamydial infections depends on the sensitivity of the techniques used. Variables include the number of body sites sampled, the number of samples obtained, and the number of passages in tissue culture. To assess these factors, microdilution plate cultures with a single blind passage were performed on specimens from 10,291 men and women attending a sexually transmitted disease clinic. Overall, 21% of the men and 30% of the women were culture positive. However, 18% of endocervical, 28% of female urethral, and 29% of male urethral cultures that were positive became so only after a single passage. Of culture-positive women, 23% were positive at the urethra only. Pooled urethral and endocervical specimens were positive more often than an endocervical specimen alone but less often than separately cultured endocervical and urethral specimens. A total of 221 specimens from 92 men and 66 women were subjected to five serial blind passages. Of 83 positive specimens, 29 (35%) were positive only after two or more passages. A total of 37 (46%) women were culture-positive, but only 12 (33%) of those who were positive and had an endocervical culture would have been detected by a single endocervical culture that was not passaged. The sensitivity of chlamydial culture is substantially less than 100% but can be improved by culturing samples from both the urethra and endocervix in women and by serial passage in tissue culture.

The prevalence of chlamydial infections is not known, although the Centers for Disease Control recently estimated that there are in excess of 3×10^6 new cases per year in the United States (1). Such estimates are based on extrapolations from the numbers of reported cases of gonorrhea and ratios of chlamydial to gonococcal infections in patients attending sexually transmitted disease clinics. Prevalence data has varied widely depending on the populations sampled (20) and the methods of detection used (11). Tissue culture isolation has become the most widely accepted method. However, its sensitivity and specificity are not known, and they appear to be subject to a number of variables. These include the method of sample collection, storage conditions, and the method of identification of organisms (11). Differences in the recovery of organisms from single as opposed to multiple samples taken from the same body site at the same time (3, 4) and in recovery from sequential samples taken from the same patient at different time intervals (14) indicate that the sensitivity of culture identification is less than 100%. In addition, *Chlamydia trachomatis* is frequently recoverd from the urethra of women at risk for infection when endocervical cultures are negative (6, 13, 22). Some of these women present with dysuria and pyuria, suggesting a diagnosis of urethritis (18, 22). However, others are asymptomatic, and their infections would go undetected if urethral cultures were not performed (13, 22).

We recently observed that when endometrial biopsy specimens were subjected to serial blind passage, there was an increase in the number of positive cultures with passage (2, 7), suggesting that multiple passage might increase recovery when the number of organisms present was low. The present study was designed to determine whether this might also be true for urogenital specimens and to determine the extent to which urethral cultures could increase the frequency of identification of infected women. The results indicate that both procedures produce substantial increases in rates of isolation from infected individuals.

MATERIALS AND METHODS

Population. During a 1-year period, all consenting men and women who were attending a sexually transmitted disease clinic and were felt to be at risk for chlamydial infection had specimens cultured for *C. trachomatis*. Endourethral specimens were obtained from both men and women, and endocervical specimens were obtained from women. Men were considered to be at risk if they had urethritis or had had sexual contact with women known to be infected. Women were considered at risk if they had gonorrhea or cervicitis or had had sexual contact with men with urethritis or known chlamydial infection. Specimens from additional patients, not in one of these risk categories, were cultured at the discretion of the nurse providing care. Of the 10,291 patients from whom specimens were cultured during this period, 5,998 were men and 4,293 were women; 8,079 fell into one of the defined risk groups, and 2,212 did not.

An additional 217 consenting women whose diagnosis placed them in one of the defined risk groups had duplicate swabs obtained from both the endocervix and urethra. After the ectocervix had been cleaned with a gauze-covered cotton applicator and an endocervical specimen had been collected for gonorrhea culture, two sequential samples were obtained from the endocervix by using Dacron-tipped plastic swabs (American Scientific Products, McGaw Park, Ill.). Two sequential specimens were obtained from the endourethra by using cotton-tipped wire swabs (Medical Wire & Equipment

* Corresponding author.

TABLE 1. Number of swab specimens from each body site positive for *C. trachomatis*

Sex and body site[a]	No. positive with indicated technique/total no. positive (%)		
	Primary inoculation	Only after single passage	Total positive/no. cultured (%)
Men, U	910/1,283 (71)	373/1,283 (29)	1,283/5,998 (21)
Women			
C	815/989 (82)	174/989 (18)	989/4,315 (23)
U	534/742 (72)	208/742 (28)	742/4,285 (17)
C or U	1,010/1,281 (79)	271/1,281 (21)	1,281/4,293 (30)

[a] Abbreviations: U, urethra; C, endocervix.

Co., Ltd., Corsham, England). For the first 2 weeks of the study, the first of the two swabs from each body site were placed together in a vial containing 1.5 ml of transport medium, and then the second swabs from each body site were placed in separate vials. During the second 2 weeks of the study, the order was reversed; the first swab obtained from each body site was placed in an individual vial of transport medium, and the second swabs from each site were combined in a single vial.

Specimens from an additional 92 men and 66 women, each of whom also had a clinical diagnosis placing them in a risk group, were subjected to multiple blind passages as described below.

Culture technique. Cultures were performed essentially as described previously (8). Briefly, specimens were stored at 4°C for less than 24 h before tissue culture inoculation or further storage at −70°C (less than 1 week). Each vial of transport medium contained, in addition to the swab or swabs, two glass beads. Before inoculation, the vial was agitated vigorously with a vortex mixer, and 0.1 ml of the specimen was used to inoculate a McCoy cell monolayer in a microdilution plate. These inoculations were done in triplicate, except where otherwise indicated. Next, 0.2 ml of tissue culture medium was added to the inoculum, and centrifugation was performed at 1,750 × *g* at 30°C for 1 h. After centrifugation, the plates were incubated at 35°C for 72 h, and then one monolayer for each specimen was assessed for inclusion formation by indirect immunofluorescence staining using a genus-reactive monoclonal antibody (8). This antibody has been shown to bind to inclusions from representative stains of each of the 15 known serovars of *C. trachomatis* and to provide an intensity of staining with a fluorescein-labeled, rabbit anti-mouse immunoglobulin G (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.) equivalent to that obtainable with commercially available antichlamydial antibodies (Chlamydia Culture Confirmation Kit; Syva Corp., Palo Alto, Calif.; data not shown). The medium was aspirated from the two unstained monolayers for each specimen, and 0.1 ml of transport medium was added to the first of these. The cells were scraped and disrupted with a blunt Pasteur pipette. The suspension was aspirated and transferred to the second well. These cells were then scraped, disrupted, and aspirated, and the pooled contents of the two wells were used to inoculate a fresh monolayer of McCoy cells in a single well. To this well, 0.2 ml of tissue culture medium was then added, and centrifugation was performed as described above. After an additional 72 h of incubation, this well was also stained and examined for inclusion formation.

For the multiple passage study, the procedure described above was modified as follows. All specimens were inoculated in triplicate within 24 h of obtainment. None was stored at −70°C. After the first 72-h incubation, one well was stained and examined for inclusions. The tissue culture medium was aspirated from the remaining two wells, 0.15 ml of transport medium was added to each well, the contents were scraped as before, and the suspensions from the two wells were pooled and used to inoculate three fresh monolayers. These monolayers were incubated for 72 h, one monolayer was stained, and suspensions from the other two were passaged to three fresh monolayers. The entire process was repeated three more times for a total of five passages. To minimize the possibility of cross contamination, these passages were done in a separate hood from those in which other chlamydia work was ongoing. In addition, control wells inoculated with sterile transport medium were randomly interspersed with specimen wells such that there was approximately one control well for two specimen wells. The initial inoculation was done by a different technician than the one who subsequently passaged and read the specimens. The technician performing the passages and evaluating the stained monolayers did not know which wells had been inoculated with sterile medium as opposed to patient specimens.

Statistical methods. Dichotomous variables were compared by using Fisher's exact test (two tailed). Comparisons involving inclusion-forming units were performed using Student's *t* test after a log transformation of the number of units, except for the comparison of the urethral and endocervical sites for women who were positive at both sites, in which case a paired *t* test was used.

RESULTS

Urethral cultures and single passage. Twenty-one percent of the men and 30% of the women were culture positive for *C. trachomatis* (Table 1). However, 18% of endocervical, 28% of female urethral, and 29% of male urethral cultures that were positive became so only after a single passage. Of the 1,281 culture-positive women, 539 (42%) were positive only at the cervix, 292 (23%) were positive only at the urethra, and 450 (35%) were positive at both sites. Endocervical cultures were more likely to be positive without passage than those from either the female or male urethra ($P < 0.001$; Table 1). This tendency paralleled the number of inclusion-forming units per well detected in those specimens which were positive on primary inoculation (i.e., without passage). The geometric mean of inclusions produced on primary inoculation by specimens from the male urethra was significantly higher than that for female urethral specimens (19.6 versus 12.6; $P = 0.0001$), and both were lower than the geometric mean of inclusions produced by endocervical specimens (42.7; $P < 0.0001$). A complaint of dysuria was elicited from only 26 (4.0%) of the women whose urethral cultures were positive for *C. trachomatis* as compared with 173 (6.5%) of those whose urethral cultures were negative ($P = 0.015$).

Comparison of combined and separate endocervical and urethral swabs from women. Of the 417 women who were sampled twice at both the endocervix and urethra, 133 (32%) had at least one positive culture. The order in which the specimens were obtained did not affect the outcome, i.e., there was no difference in the recovery of the organisms whether the combined swabs were the first or second obtained (Table 2). Of the 133 positive women, 91 (68%) were detected with a single endocervical specimen and an additional 26 (19%) had positive urethral but negative endocer-

TABLE 2. Comparison of culture of combined and separate endocervical and urethral swabs from women

Culture(s) and result(s)[a]	No. positive (% of total positive)[b]		
	Combined 1st	Combined 2nd	Total
C+	49 (71)	42 (65)	91 (68)
C−, U +	12 (17)	14 (22)	26 (19)
C−, U−, C/U+	8 (12)	8 (12)	16 (12)
C/U+	53 (77)	51 (80)	104 (78)
C−, C/U+	14 (20)	17 (27)	31 (23)
Total positive	69 (100)	64 (100)	133 (100)

[a] Abbreviations: U, urethra; C, endocervix.
[b] Combined 1st means that the first swabs taken at each site were combined into a single vial (abbreviated C/U) and the 2nd swab taken at each site was placed in an individual vial; for combined 2nd, the converse was true.

vical cultures. The combined results from these separately cultured specimens identified as infected 117 women, or 88% of the total. The specimens in which swabs from both sites had been pooled into a single vial were positive for 104 (78%) of the 133 infected women. The combined swab specimen was positive for 31 women with negative endocervical cultures. Of these, 16 (12% of the total) also had negative urethral cultures and their infections would not have been detected had the combined swab specimens not been obtained in addition to the separate site specimens. The combined specimen was less sensitive (78%) in detecting infected women than were the combined results of separate cultures of samples from the cervix and urethra (88%) but more sensitive than was a single endocervical culture (68%).

Effect of serial blind passage on recovery. A total of 221 swab specimens were subjected to five serial blind passages. Eighty-three (38%) were eventually positive. Of these, 29 (35%) were positive without passage and an additional 24 (total of 53 or 64%) were positive after a single passage. Of the positive specimens, 29 (35%) were identified as such only after two or more passages. Two (2%) of the 101 randomly assigned control specimens were falsely positive, one after three passages and one after four passages. When an isolate was positive only after passage, typically only a few inclusions were seen the first time it was positive, followed by increases with additional passages. The results by risk category and the passage number at which a specimen (or specimens) first became positive are given for men in Table 3 and for women in Table 4. Three women had specimens cultured from the urethra but not the endocervix because of the surgical absence of the latter. One of these cultures was positive after three passages. A total of 37 women were

TABLE 3. Recovery of *C. trachomatis* from urethral swabs from men

Risk group (no. tested)[a]	No. of specimens first positive at passage no.:						Total % positive
	0	1	2	3	4	5	
NGU (43)	6	5	3	2	0	0	37
GC (34)	1	4	1	1	1	0	24
GC contact (12)	1	0	3	0	1	0	42
CT contact (3)	1	0	0	0	0	0	33
All (92)	9	9	7	5	2	0	33
Cumulative % positive	30	60	83	93	100	100	

[a] Abbreviations: NGU, nongonococcal urethritis; GC, gonorrhea; CT, chlamydia.

TABLE 4. Recovery of *C. trachomatis* from urethral or endocervical swabs from women

Risk group (no. tested)[a]	No. of specimens first positive at passage no.:						Total % positive
	0	1	2	3	4	5	
NGU contact (23)	3	3	3	3	0	0	52
GC (9)	2	3	0	0	0	0	56
GC contact (22)	5	3	2	0	1	0	50
CT contact (4)	2	0	0	0	0	0	50
Cervicitis (8)	3	1	1	1	0	1	88
All (66)	15	10	6	4	1	1	56
Cumulative % positive	41	68	84	95	97	100	

[a] Abbreviations are defined in Table 3, footnote *a*.

positive at one or both sites. However, only 12 (33%) of the 36 women who were culture positive and had endocervical cultures performed had positive endocervical cultures without passage. Eight additional women had positive urethral cultures (three in the initial well and five after passage), but negative (seven) or no (one) endocervical cultures, and another 17 had positive endocervical cultures only after passage.

DISCUSSION

The data indicate that the sensitivity of a single culture for *C. trachomatis* as it is usually obtained is considerably less than 100%. If it is assumed that the endocervical and urethral cultures with multiple passages correctly identified all infected women in the multiple passage portion of the study (which is unlikely), then the sensitivity of a single endocervical specimen inoculated into a single microdilution well and not passaged would have been only 33%, since only 12 of the culture-positive women had positive endocervical cultures without passage. The women who had positive urethral cultures but negative endocervical cultures would have been missed, as would the women whose endocervical cultures were positive only after passage. Multiple passage data, analogous to that presented here, has not been previously reported. However, in other single-passage studies using immunofluorescence for detection, a far smaller porportion of infected individuals was identified only after passage (17, 19, 23) than was observed after one passage in the present study. The reason for this discrepancy is not readily apparent, although one important difference in technique may have been the passage of two wells to one in single passage, as opposed to the passage of one well to one, which is the more usual procedure.

The isolation rates for the specimens subjected to multiple passages were relatively low after no, or only one, passage for patients within the risk groups tested. However, the rates for these patients were not low after multiple passages. This raises the possibility that in this particular set of experiments some isolates that were detected only after multiple passes might have been detected earlier in another set of experiments. However, the experiments reported represent a series of four separate inoculations over a 2-month period of time, during which standard inocula of control organisms were readily identified. It is also quite unlikely that the positive cultures detected after passage in these experiments resulted from cross contamination. Cross contamination can occur with multiple passage and was a problem in some of our early experiments with this technique (data not shown). However, with observer-blind passage and reading, it was

less than 2% for the control wells in the experiments reported here, and when it occurred, it always did so after three or more passes.

We do not know why some isolates produce visible inclusions in a microdilution system only after passage. Persistent infection of tissue culture monolayers with both *Chlamydia psittaci* (12) and *C. trachomatis* (9) has been described, and evidence was derived for a cryptic form of the former but not the latter (9). It may be that some proportion of infectious particles in a clinical specimen are simply unable to establish productive infection until they have undergone some type of modification, either by the host cells (tissue culture in this case) or as a result of activation of some sort of intrinsic trigger mechanism. If the proportion of such particles was on the same order as the proportion of inclusion-forming units, then specimens containing low total numbers of chlamydia particles would have a higher probability of requiring passage before forming inclusions than would specimens containing many organisms. Such an explanation is consistent with the observation that specimens (e.g., endocervical) which are more frequently positive without passage also produce more inclusions when they are positive.

Also, it is quite possible that this phenomenon may occur infrequently in other culture systems, such as vial culture. Vial culture permits a larger total inoculum and offers a larger surface area for infection. Both of these factors should increase the probability in a given specimen that an infectious particle capable of causing infection will come in contact with a susceptible cell. In fact, it has been shown in single-passage experiments that vial culture is more likely to detect infectious particles without passage than microdilution well culture is (15). However, culture in vials is less suitable for processing large numbers of clinical specimens, and as a result, chlamydia culture in microdilution plates has become a common practice. Consequently, the observations reported here are relevant to this system. The extent to which they prove relevant to other systems, such as vial culture, will depend on the results of future experiments.

The increased identification of infected women as a result of urethral cultures remained relatively constant at 19 to 23%, whether the determination was made after primary inoculation, single passage, or multiple passages. Whether this represented urethral infection in the absence of endocervical infection or simply reflected the lack of sensitivity of endocervical culture remains to be determined. The infrequency with which a complaint of dysuria was associated with chlamydia urethral infection in women indicates that, although this condition may produce symptoms (18), it usually does not. Similar observations have been reported by others (22).

Since urethral infection in women is often associated with endocervical infection, the social and therapeutic implications of a positive urethral culture are the same as those of a positive endocervical culture. Consequently, an attempt was made to combine urethral and endocervical specimens and process them together. These combined cultures were somewhat less sensitive in detecting chlamydial infection than were cultures when the two specimens were inoculated separately. This may have been due to less efficient dispersion of chlamydial particles from the swabs into the medium in vials containing two swabs, as opposed to one swab. However, the combined swabs did detect more infected women than did a single endocervical swab. In addition, the combined specimens identified an additional 16 (12%) infected individuals not detected by the separate swabs alone. This observation is consistent with previous reports that multiple sampling from the same site results in an increased isolation rate (3, 4).

Unfortunately, the data do not provide an absolute measure of the sensitivity of tissue culture isolation for *C. trachomatis*, but only an indication that it is considerably less sensitive than has been suggested (14). They also indicate that recovery can be improved in women by performing both endocervical and urethral cultures and in both men and women by blind passage. Although multiple passage may be impractical for most clinical specimens, a single blind passage can substantially increase recovery with a minimal increase in cost.

In addition, the lack of sensitivity of culture, especially when no blind passage is performed, must be considered in the evaluation of direct antigen tests for the detection of chlamydial infections. Available direct antigen tests are less sensitive than culture as it is usually performed (5, 10, 16, 21). Consequently, they will detect even fewer infected patients than will culture, and a negative direct antigen test (or culture) does not necessarily indicate the absence of chlamydial infection. Thus, any negative test for *C. trachomatis* urogenital infection must be interpreted with considerable caution.

Finally, the prevalence of infection in the women evaluated by multiple passage was quite high (56%). If these observations can be confirmed in larger numbers of patients and extended to other risk groups, they will necessitate a reevaluation of current thought regarding the prevalence and epidemiology of chlamydial infections of the urogenital tract.

ACKNOWLEDGMENTS

We thank Barbara Ray and the staff of the Bell Flower Clinic for their assistance in enrolling patients in this study.

This work was supported by a 318(b) grant from the Centers for Disease Control and by Public Health Service grant P01 AI 20110 from the National Institutes of Health.

LITERATURE CITED

1. **Centers for Disease Control.** 1985. *Chlamydia trachomatis* infections: policy guidelines for prevention and control. Morbid. Mortal. Weekly Rep. **34**(Suppl.):53S–74S.
2. **Cleary, R. E., and R. B. Jones.** 1985. Recovery of *Chlamydia trachomatis* from the endometrium in infertile women with serum antichlamydial antibodies. Fertil. Steril. **44**:233–235.
3. **Dunlap, E. M. C., B. T. Goh, S. Darougar, and R. Woodland.** 1985. Triple-culture tests for diagnosis of chlamydial infection of the female genital tract. Sex. Transm. Dis. **12**:68–71.
4. **Goh, B. T., E. M. C. Dunlap, S. Darougar, and R. Woodland.** 1985. Three sequential methods of collecting material from the urethra of men for culture for *Chlamydia trachomatis*. Sex. Transm. Dis. **12**:173–176.
5. **Howard, L. V., P. F. Coleman, B. J. England, and J. E. Herrmann.** 1986. Evaluation of Chlamydiazyme for the detection of genital infections caused by *Chlamydia trachomatis*. J. Clin. Microbiol. **23**:329–332.
6. **Johannisson, G., G.-B. Lowhagen, and E. Lycke.** 1980. Genital *Chlamydia trachomatis* infection in women. Obstet. Gynecol. **56**:671–675.
7. **Jones, R. B., J. B. Mammel, M. K. Shepard, and R. R. Fisher.** 1986. Recovery of *Chlamydia trachomatis* from the endometrium of women at risk for chlamydial infection. Am. J. Obstet. Gynecol. **155**:35–39.
8. **Jones, R. B., R. A. Rabinovitch, B. P. Katz, B. E. Batteiger, T. S. Quinn, P. Terho, and M. A. Lapworth.** 1985. *Chlamydia trachomatis* in the pharynx and rectum of heterosexual patients at risk for genital infection. Ann. Intern. Med. **102**:757–762.
9. **Lee, C. K., and J. W. Moulder.** 1981. Persistent infection of mouse fibroblasts (McCoy cells) with a trachoma strain of

Chlamydia trachomatis. Infect. Immun. **32**:822–829.
10. **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.
11. **Mardh, P.-A., L. Westrom, S. Colleen, and P. Wolner-Hanssen.** 1981. Sampling, specimen handling, and isolation techniques in the diagnosis of chlamydia and other genital infections. Sex. Transm. Dis. **8**:280–285.
12. **Moulder, J. W., N. J. Levy, and L. P. Schulman.** 1980. Persistent infection of mouse fibroblasts (L cells) with *Chlamydia psittaci*: evidence for a cryptic chlamydial form. Infect. Immun. **30**:874–883.
13. **Paavonen, J.** 1979. *Chlamydia trachomatis*-induced urethritis in female partners of men with nongonococcal urethritis. Sex. Transm. Dis. **6**:69–71.
14. **Schachter, J.** 1984. Biology of *Chlamydia trachomatis*, p. 243–257. *In* K. K. Holmes, P.-A. Mardh, P. F. Sparling, and P. J. Wiesner (ed.), Sexually transmitted diseases. McGraw-Hill Book Co., New York.
15. **Schachter, J.** 1985. Immunodiagnosis of sexually transmitted disease. Yale J. Biol. Med. **58**:443–452.
16. **Stamm, W. E., H. R. Harrison, E. R. Alexander, L. D. Cles, M. R. Spence, and T. C. Quinn.** 1984. Diagnosis of *Chlamydia trachomatis* infections by direct immunofluorescence staining of genital secretions, a multicenter trial. Ann. Intern. Med. **101**:638–641.
17. **Stamm, W. E., M. Tam, M. Koester, and L. Cles.** 1983. Detection of *Chlamydia trachomatis* inclusions in McCoy cell cultures with fluorescein-conjugated monoclonal antibodies. J. Clin. Microbiol. **17**:666–668.
18. **Stamm, W. E., K. F. Wagner, R. Amsel, E. R. Alexander, M. Turck, G. W. Counts, and K. K. Holmes.** 1980. Causes of the acute urethral syndrome in women. N. Engl. J. Med. **303**:409–415.
19. **Stephens, R. S., C.-C. Kuo, and M. R. Tam.** 1982. Sensitivity of immunofluorescence with monoclonal antibodies for detection of *Chlamydia trachomatis* inclusions in cell culture. J. Clin. Microbiol. **16**:4–7.
20. **Thompson, S., and E. Washington.** 1983. Epidemiology of sexually transmitted *Chlamydia trachomatis*. Epidemiol. Rev. **5**:96–123.
21. **Tjiam, K. H., B. Y. M. van Heijst, A. van Zuuren, J. H. T. Wagenvoort, T. van Joost, E. Stolz, and M. F. Michel.** 1986. Evaluation of an enzyme immunoassay for the diagnosis of chlamydial infections in urogenital specimens. J. Clin. Microbiol. **23**:752–754.
22. **Wallin, J. E., S. E. Thompson, A. Zaidi, and K.-H. Wong.** 1981. Urethritis in women attending an STD clinic. Br. J. Vener. Dis. **57**:50–54.
23. **Zapata, M., M. Chernesky, and J. Mahony.** 1984. Indirect immunofluorescence staining of *Chlamydia trachomatis* inclusions in microculture plates with monoclonal antibodies. J. Clin. Microbiol. **19**:937–939.