

Ability of the Digene Hybrid Capture II Test To Identify *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Cervical Specimens

JULIUS SCHACHTER,^{1*} EDWARD W. HOOK III,² WILLIAM M. McCORMACK,³ THOMAS C. QUINN,⁴
MAX CHERNESKY,⁵ SYLVIA CHONG,⁵ JENNIFER I. GIRDNER,⁴ PAULA B. DIXON,²
LYNETTE DEMEO,³ EVA WILLIAMS,¹ ALLISON CULLEN,⁶ AND ATTILA LORINCZ⁶

*Chlamydia Research Laboratory, Department of Laboratory Medicine, University of California, San Francisco, San Francisco, California*¹; *Division of Infectious Diseases, University of Alabama, Birmingham, Alabama*²; *Division of Infectious Diseases, State University of New York, Brooklyn, New York*³; *Division of Infectious Diseases, Johns Hopkins University, Baltimore*,⁴ *and Digene Corporation, Silver Spring*,⁶ *Maryland; and St. Joseph's Hospital, McMaster University, Hamilton, Ontario, Canada*

Received 17 March 1999/Returned for modification 1 June 1999/Accepted 9 August 1999

The Digene Hybrid Capture II (HCII CT/GC) test is a combination test designed to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in a single specimen. It is a nucleic acid hybridization test which uses signal amplification to increase sensitivity. We compared its performance to that of culture on cervical specimens from 1,370 women. Direct fluorescent-antibody assay was used to resolve discrepant results for *C. trachomatis*. Samples were collected with a proprietary cervical brush or with endocervical swabs. The HCII CT/GC test proved to be sensitive and specific in detecting these organisms. Compared to *N. gonorrhoeae* culture, it had a sensitivity of 93% (87/94) and a specificity of 98.5% (1,244/1,263). Compared to *C. trachomatis* culture, the sensitivity was 97.7% (129/132) and specificity was 98.2% (1,216/1,238). Testing of some specimens with discrepant results by PCR suggested that the test would actually prove to be even more specific if it were compared to a nucleic acid amplification test (NAAT). The sensitivity of *C. trachomatis* culture was somewhat less, at 88.6% (117/132). The endocervical brush appeared to be better than Dacron swabs for collecting specimens. The HCII CT/GC test offers an attractive format that allows simultaneous detection of *C. trachomatis* and *N. gonorrhoeae* with a single specimen. An initial positive result is followed by repeat tests with probes to identify chlamydiae or gonococci. This test is more sensitive than *C. trachomatis* culture and is at least as sensitive as culture for gonococci. It deserves further evaluation and comparison with NAATs and may well offer an attractive alternative for diagnosis and screening of these infections.

Chlamydia trachomatis and *Neisseria gonorrhoeae* are the two most common sexually transmitted bacterial pathogens (1). They are responsible for a wide variety of acute clinical conditions and for important long-term consequences that adversely affect women's reproductive health. They threaten the health of newborn infants as well as adults. Although some progress has been made in reducing the prevalence of these infections, they still are occurring at epidemic rates in many parts of the world. Successful control of these infections is based in large part on screening and treatment of asymptomatic individuals as well as appropriate management of symptomatic men and women. Accurate diagnostic tests are needed for the management of acute conditions and are required for the detection of infections in asymptomatic individuals.

Culture, both for gonococci and chlamydiae, has long been considered the diagnostic test of choice. Nucleic acid amplification tests (NAATs) that are slightly more sensitive than culture for gonococci when culture is performed under good conditions have been introduced (6). Unfortunately, culture sensitivity is lower when specimens have to be transported to the laboratory, as success depends on strict maintenance of cold chains and use of appropriate transport media. The

NAATs are relatively much more sensitive for diagnosing chlamydial infection than they are for diagnosing gonococcal infection. This is because the sensitivity of chlamydial culture is lower and culture performance is more variable from laboratory to laboratory than is culture for gonococci (2, 4, 7).

Culture for chlamydiae is labor intensive, time consuming, and costly. While direct antigen detection methods, enzyme immunoassays, and direct fluorescent-antibody assays (DFA) are more rapid and less expensive, they are also less sensitive than culture in quality laboratories (9). While NAATs are more sensitive and more specific and allow testing of urine and vaginal swab specimens, there is still a need for rapid, less expensive, accurate diagnostic tests for chlamydiae and gonococci. The Digene Hybrid Capture II (HCII CT/GC) test is a nucleic acid probe-based chemiluminescent assay that will detect chlamydial or gonococcal DNA in cervical specimens (8). The target DNA is hybridized with RNA probes. The hybrids are immobilized in an antibody capture system on microtiter plates. Rather than amplifying the target, as is done in NAATs, the HCII system uses a signal amplification method.

We assessed the performance of an investigational combination test (HCII CT/GC) designed to detect the presence of chlamydiae and gonococci in a single endocervical specimen by comparing its performance to those of cultures for these microorganisms. Although the Digene HCII CT/GC test can be used for diagnosis of single-pathogen infections, the test format also allows simultaneous detection of both *N. gonorrhoeae* and *C. trachomatis*, or either, in the specimen, followed by

* Corresponding author. Mailing address: Chlamydia Research Laboratory, Department of Laboratory Medicine, University of California, San Francisco, 1001 Potrero Ave., SFGH 3416, San Francisco, CA 94110. Phone: (415) 824-5115. Fax: (415) 821-8945. E-mail: jsch@itsa.ucsf.edu.

specific identification. Thus, the results of our evaluation will be presented in that sequence—first the combined results, then the single-pathogen identification. Because it is recognized that chlamydial culture is less than 100% sensitive, specimens that were positive for chlamydial DNA by the Digene test but negative by culture had the remainder of the tissue culture transport medium spun and the sediment tested by DFA for chlamydial antigen.

MATERIALS AND METHODS

This study was a multicenter trial performed at five sites in the United States: University of Alabama at Birmingham (UAB), Birmingham, Ala.; University of California San Francisco (UCSF), San Francisco, Calif.; Johns Hopkins University (JHU), Baltimore, Md.; and State University of New York (SUNY), Brooklyn, N.Y. Women were seen in either sexually transmitted disease clinics or family planning clinics. The study was approved by the respective university ethical review boards, and the patients gave informed consent and were ≥ 18 years old. Those who had received antimicrobial therapy within the last 21 days were excluded. The McMaster University site (Hamilton, Ontario, Canada) participated only in the evaluation of Dacron swabs for specimen collection, while the other sites evaluated the Dacron swab and the Digene cervical brush.

Specimen collection. A total of three endocervical specimens were collected from each participant. For culture, two specimens were collected with Dacron swabs. At SUNY some specimens for chlamydia culture were collected with Calgiswabs. For the Digene HCII CT/GC test, a proprietary endocervical brush called a cervical sampler was used to collect specimens from nonpregnant women, while a Dacron swab was used to collect specimens from pregnant women. The order of specimen collection was as follows: the first swab was for gonococcal culture, and the swab for the chlamydial culture and the sample collection for the Digene HCII CT/GC test were done in random order. Prior to the collecting of specimens, the exocervix was cleaned of mucous and exudate, after which the swab or cervical sampler was rubbed against the endocervix and removed, with contact with any vaginal surface being avoided. An additional subset of women was evaluated with a swab, instead of the proprietary Digene brush, being used for collection of the endocervical specimen.

Culture methods. Gonococci were identified by culture on Thayer-Martin plates (Remel Inc., Lenexa, Kans.). Oxidase-positive, gram-negative diplococci were confirmed as gonococci by use of sugar utilization tests, except at SUNY, where Gonocheck-II (EY Laboratories, San Mateo, Calif.) was used. *C. trachomatis* was isolated in McCoy cell culture with centrifugation of the inoculum with cycloheximide-treated cells and DFA stain (Syva MicroTrak culture confirmation reagent; Behring Diagnostics, Cupertino, Calif.) to identify inclusions (9). Cervical swab specimens were refrigerated from the time of collection until inoculation within 72 h of collection, except in Brooklyn where specimens were frozen at -80°C . Each laboratory used its own standard procedure. The use of 1-dram shell vials with a blind passage was routine in San Francisco; the use of the vial but no blind pass was standard at the Brooklyn and Hamilton laboratories; and the use of 96-well microtiter plates, with a pass, was standard at the Birmingham and Baltimore laboratories.

Digene HCII CT/GC test specimen processing. Specimens were held at 4°C and processed within 7 days of collection or frozen (up to 3 weeks) until tested. Denaturation reagent (500 μl) was added to each specimen-containing tube in 1 ml of a transport medium. The tubes were then vortexed at high speed for 5 s, inverted, and incubated at 65°C for 45 min.

Digene HCII CT/GC test procedure. The manufacturer's instructions were followed. Treated specimens and controls (75 μl) were placed in a microtube along with 25 μl of the RNA probe mix that targets the genomic and cryptic plasmid DNA sequences of the organisms. The covered microtubes were placed on a rotary shaker for 5 min and then incubated at 65°C for 60 min. After hybridization the microtube contents were transferred to microwells for capture of the hybrid product. They were placed on the rotary shaker (1,100 rpm) for 60 min, and then the wells were emptied and the fluids were discarded. To detect the captured hybrids, 75 μl of a proprietary detection reagent was added to each well, and the plates were incubated for another 30 min. The detection reagent contains an antibody conjugate specific for RNA-DNA hybrids. Multiple enzymes are conjugated to each antibody molecule, and many antibodies bind to each RNA-DNA hybrid, resulting in a marked amplification of the signal. The plates were again emptied and allowed to sit inverted on absorbent paper for 1 to 2 min. The wells were then manually washed six times with buffer. After washing, the plates were inverted onto absorbent paper and drained for 5 min. Then, to generate a signal, 75 μl of a chemiluminescent reagent was added to each well and the test specimens were incubated at room temperature for 15 min. The plates were read on an MLX or ML2200 luminometer (Dynex Technologies, Chantilly, Va.). The cutoff for a positive test was the mean relative light units generated by a series of three positive controls. An initial positive result meant that the clinical specimen was testing positive for either *C. trachomatis* or *N. gonorrhoeae*, or both. A second HCII test (Digene HCII CT-ID or GC-ID) was performed on specimens that yielded an initial positive result to identify whether that initial signal was due to the presence of chlamydial or gonococcal DNA.

TABLE 1. HCII CT/GC test results^a compared to those of culture for gonococci and culture or DFA for chlamydiae

HCII CT/GC test result	No. of specimens	
	Positive ^b	Negative ^c
Positive	193	25
Negative	9	1,115

^a Results compared for cervical brush-collected specimens only. $n = 1,342$; prevalence = 15.1%; sensitivity = 95.5%; specificity = 97.7%.

^b Positive by culture for chlamydiae or culture for gonococci or negative by culture but positive by DFA for chlamydiae.

^c Negative by culture for gonococci or by both culture and DFA for chlamydiae.

Thus, specimens that were positive had the testing sequence repeated with separate sets of specific probes for chlamydial and gonococcal DNA.

Discrepant analysis. To further evaluate specimens that yielded apparent false-positive results (i.e., those that were negative for chlamydiae by culture but positive by the Digene HCII CT-ID test), tissue culture transport medium remnants were cytospun ($14,000 \times g$ for 15 min) and a DFA stain was performed on the sediment with the Syva MicroTrak *C. trachomatis* DFA. The criterion for a positive result was two or more elementary bodies. Where possible, specimens that were negative by DFA were further tested by PCR (AMPLICOR; Roche Molecular Diagnostics, Nutley, N.J.). To evaluate the specimens that yielded apparent false-positive results in tests for gonococci a PCR test was done (5) on DNA purified by phenol-ethanol extraction from the denatured Digene test specimen remnants.

RESULTS

Overall performance of HCII CT/GC test. A total of 1,370 women were enrolled in the HCII CT/GC test evaluation where the cervical brush was the collection device. Of these women, 1,342 had all tests performed. Due to cell culture toxicity, broken specimens, etc., the number that was evaluated for chlamydia or gonorrhea is not the same as the total number recruited or the number having all tests performed. The results, by study site and clinical status, will be presented in greater detail below, as will the specific results for identification of chlamydial infection or gonorrhea.

There were 218 HCII CT/GC test results that were positive (Table 1). Of these, 193 specimens were positive by culture for either gonorrhea or chlamydia or positive by DFA for chlamydia. There were nine specimens positive by culture but not by the HCII CT/GC test. Thus, the sensitivity of HCII CT/GC test compared to culture and DFA was 95.5% (193/202) and the specificity was 97.8% (1,115/1,140). As shown in Table 2, despite wide ranges in prevalence there were no marked differences from laboratory to laboratory and the overall sensitivity was quite similar for symptomatic or asymptomatic women (95.2 versus 97.2%) and the specificities were virtually identical at 97.8 and 97.7%, respectively.

Ability of HCII system to detect single pathogens. (i) *N. gonorrhoeae*. Of the 218 specimens that yielded positive results in the initial HCII CT/GC test (Table 1), less than half (106) were positive for gonococcal DNA in the specific HCII GC-ID probe confirmation test. The comparison of the HCII GC-ID and gonococcal culture results is presented in Table 3. The prevalence of gonorrhea was 6.9%, the sensitivity was 92.6% (87/94), and the specificity was 98.5%.

(ii) *C. trachomatis*. Of the 218 specimens that were positive upon initial screening, 151 were positive in the HCII CT-ID test. The performance of the HCII system in diagnosing chlamydial infection as compared to chlamydia isolation in cell culture and positive DFA results is presented in Table 4. The 97.7% (129/132) sensitivity of the HCII CT-ID test was somewhat better than that of culture (88.6% [117/132]). The prevalence was 9.6%, and the specificity was 98.2%.

TABLE 2. HCII CT/GC test performance characteristics by clinical status and study site^a

Patient type and study site	No. of specimens	Prevalence (%)	Sensitivity (%)	Specificity (%)
Symptomatic				
UAB	351	21.9	97.4	97.1
JHU	187	15.0	85.7	97.5
SUNY	220	21.4	95.7	97.1
UCSF	164	7.9	100	100
Total	922	17.9	95.2	97.8
Asymptomatic				
UAB	101	13.9	100	97.7
JHU	12	25.0	100	100
SUNY	81	4.9	100	98.7
UCSF	226	6.6	93.3	97.2
Total	420	8.6	97.2	97.7

^a Cervical brush used to collect all specimens.

Ability of HCII CT/GC system to diagnose double infections.

There were 37 specimens that were positive in the specific HCII tests for both *C. trachomatis* and *N. gonorrhoeae*. Of these, 28 (75.7%) were positive by culture and/or DFA for chlamydiae and 32 (86.5%) were positive by culture for gonococci. There were 30 specimens that were positive for both gonococci and chlamydiae by culture and/or DFA; by the HCII system, 28 (93.3%) of these were positive for chlamydiae, and 27 (90%) were positive for gonococci. One specimen that was dual positive by culture was negative in the HCII CT/GC test, and one specimen that was positive in all HCII tests was negative for both pathogens by culture.

Performance of HCII system with specimens collected by swabs. All of the results presented above were obtained with cervical specimens collected with the use of a cervical brush. Dacron swabs were used instead of the brush to collect cervical specimens on an additional 247 women. The specimens were tested by the same protocols described above. Some differences in sensitivity were seen. Among the 247 women who were tested for gonorrhea, the prevalence was found to be 9.3% by culture. The sensitivity of the HCII CT/GC test followed by the HCII GC-ID test was 87%. This value was not statistically different from the 92.6% shown in Table 3 for specimens collected with the cervical brush. However, with *C. trachomatis* the sensitivity of the HCII system for the 238 specimens (prevalence, 8.8% by culture plus DFA) was 81%, which was significantly less than the 97.7% shown in Table 4 for specimens collected with the cervical brush (Fisher's exact test, $P = 0.007$).

PCR on discordant specimens. Some of the specimens that were positive by the HCII CT-ID and GC-ID tests but negative

TABLE 3. HCII GC-ID test results, showing ability to identify *N. gonorrhoeae*^a

HCII GC-ID test result	No. of specimens	
	Positive ^b	Negative ^b
Positive	87	19
Negative	7	1,244

^a $n = 1,357$; prevalence = 6.9%; sensitivity = 92.6%; specificity = 98.5%.

^b Result of culture for gonococci.

TABLE 4. HCII CT-ID test results, showing ability to identify *C. trachomatis*^a

HCII CT-ID test result	No. of specimens	
	Positive ^b	Negative ^b
Positive	129 ^c	22
Negative	3	1,216

^a $n = 1,370$; prevalence = 9.6%; sensitivity = 97.7%; specificity = 98.2%.

^b Result of culture and DFA for chlamydiae.

^c One hundred fourteen culture-positive specimens and fifteen culture-negative but DFA-positive specimens.

by culture or DFA were available for further analysis. PCR for gonococcal DNA was performed on 7 of the 19 specimens with discordant results identified in Table 3, and 6 of them were positive. Accepting the 6 specimens as true positives results in slight improvements in sensitivity (92.6 to 93.0%) and specificity (98.5 to 99.0%). Similarly, there were 22 specimens with discordant results for chlamydia, and PCR was performed on 21 of them, with 16 being positive for chlamydial DNA. The sensitivity (97.7 to 98%) and specificity (98.2 to 99.5%) improve slightly upon recalculation, counting the 16 as true positives.

DISCUSSION

The HCII CT/GC test proved to be sensitive for detection of cervical infection with *C. trachomatis* or *N. gonorrhoeae*. It offered the advantage of being able to detect the presence of both organisms in a single test. As the test is configured, the initial positive signal is the trigger for a second test to identify the specific organism responsible for the initial positive one. Thus, one can test for the presence of chlamydia and gonorrhea using a test that takes 4 h to perform, and if a positive result is obtained, one can identify whether the infectious agent is chlamydia or gonococcus in another 4-h test. The general format of this test is a strength. That the initial result is for the presence of either of two pathogens provides benefits. In the majority of settings, the great proportion of tests performed will be negative for both pathogens. Thus, there is the potential for efficiency and economy of reagents and personnel. This could permit a more rapid turnaround of results and lower costs, as the number of tests required to confirm positive results would be fewer than the number that would be required if separate tests were performed. For example, if the prevalence of these two sexually transmitted diseases is 10 to 20%, the total number of tests performed would be 55 to 60% of the number required if two single tests were performed.

The HCII CT-ID and GC-ID tests were reasonably specific, at approximately 98% for either organism. It is likely that this specificity is an underestimation. Subsequent PCR testing of some of the apparent false-positive results showed that most actually contained chlamydial or gonococcal genes. The true specificity is probably >99%. It is clear that the HCII CT/GC test must be further evaluated in direct comparisons with NAATs that are more sensitive than culture.

In this evaluation it was impossible to determine whether the HCII CT/GC test is more sensitive than gonococcal culture, since that was the only other test performed on all specimens and additional testing was not performed on all of the specimens with apparent false-positive results. If most of them actually were positive for gonococcal DNA, then the test would be marginally more sensitive than gonococcal culture. This would be similar to the results obtained by ligase chain reaction (6).

In the current evaluation, where culture or DFA was used as the "gold standard" for chlamydial infection, the HCII CT/GC test was approximately 10% more sensitive than culture. The sensitivity of culture for chlamydiae on single specimens from women is at best in the 85 to 90% range. These studies were performed in expert laboratories with what is likely to be a higher culture sensitivity than would be seen in a routine clinical lab. Thus, this nonculture format would undoubtedly perform better in most settings where culture is performed. Certainly a nonculture test will be used more widely than will culture.

Because most of the specimens with apparent false-positive results could be shown to have chlamydial or gonococcal DNA, the specificity is likely to be higher than the 97.7% obtained in this study. Thus, it is likely that this is a test that could easily be used for screening low-prevalence populations. What is important for specificity calculations is the recognition that culture is clearly an inadequate gold standard for such evaluations. The addition of DFA for *C. trachomatis* improves the situation, but it still is not adequate for a final determination. The PCR results for a subset of those specimens that were positive by the HCII CT/GC screen but negative by culture leads to the inference that the specificity of the HCII CT/GC test is actually better than what we have calculated based on the use of just culture and DFA. Of course this is an extremely important issue when screening low- to moderate-prevalence populations. Our results suggest that the test will provide adequate specificity.

It is impossible to determine the true sensitivity of the HCII CT/GC test for *C. trachomatis* in this evaluation beyond saying that it is more sensitive than culture. Further evaluation of this signal amplification test is needed, especially by direct comparison to the target NAATs that have also been found to be far more sensitive than culture. The HCII CT/GC test, based on hybridization and signal amplification, is based on a different principle than the three commercially available NAATs, which are based on amplifying target or probe.

The HCII CT/GC test format is quite reproducible, as its performance varied little from site to site. There was also very little difference in test results between patients who were symptomatic versus asymptomatic.

Although not performed with the same patients, the sensitivity of the HCII CT/GC test with specimens collected with a swab compared to that with specimens collected with a cervical brush appears somewhat lower. The results for diagnosis of gonococcal infections were not statistically different with the two specimen types. However, with chlamydial infection, the performance with the endocervical brush was statistically superior in terms of sensitivity. Brushes typically collect more epithelial cells than do swabs, and the squamocolumnar epithelial cells within the transitional zone represent the site that *C. trachomatis* infects. Culture results are acutely dependent on sampling adequate epithelial cells, as are DFA results. It would have been more desirable from a screening viewpoint if the swab- and brush-collected specimens yielded equivalent per-

formances because brushings are contraindicated during pregnancy and pregnant women are one of the priority groups for screening for chlamydial and gonococcal infections. It should be stressed that even though swabs appear to be somewhat less sensitive for specimen collection with this test, the sensitivity of the HCII CT/GC test with the swab-collected specimens was still higher than the sensitivity of culture and predictably would be higher than either antigen detection or RNA hybridization without amplification (3) assays. Further studies are needed to better define the effect of collection device on test performance.

In summary, the HCII CT/GC test is relatively easy to perform and provides a nonculture method of diagnosing chlamydial and gonococcal infections that is of similar sensitivity to gonococcal culture and more sensitive than chlamydial culture. The ability to detect chlamydial and gonococcal infection with a single test is an attractive option. Urethral swabs and urine specimens from men, and vaginal swabs and urine from women, need to be evaluated for performance before the ultimate usefulness of the HCII CT/GC test in screening or diagnosis can be determined. Further evaluation in direct comparison with target NAATs is clearly called for. If the economics are attractive the HCII CT/GC test could offer a reasonable alternative to other methods of diagnosing chlamydial and gonococcal infections.

REFERENCES

1. Centers for Disease Control and Prevention. 1998. 1998 Guidelines for treatment of sexually transmitted diseases. *Morbidity and Mortality Weekly Report* 47(RR-1):1-111.
2. Chernesky, M. A., 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.
3. Clarke, L. M., M. F. Sierra, B. J. Daidone, N. Lopez, J. M. Covino, and W. M. McCormack. 1993. Comparison of the Syva MicroTrak enzyme immunoassay and Gen-Probe PACE 2 with cell culture for diagnosis of cervical *Chlamydia trachomatis* infection in a high-prevalence female population. *J. Clin. Microbiol.* 31:968-971.
4. Crotchfelt, K. A., B. Pare, C. Gaydos, and T. C. Quinn. 1998. Detection of *Chlamydia trachomatis* by the Gen-Probe AMPLIFIED Chlamydia Trachomatis Assay (AMP CT) in urine specimens from men and women and endocervical specimens from women. *J. Clin. Microbiol.* 36:391-394.
5. Ho, B. S., W. G. Feng, B. K. Wong, and S. I. Egglestone. 1992. Polymerase chain reaction for the detection of *Neisseria gonorrhoeae* in clinical samples. *J. Clin. Pathol.* 45:439-442.
6. Hook, E. W., III, S. F. Ching, J. Stephens, K. F. Hardy, K. R. Smith, and H. H. Lee. 1997. Diagnosis of *Neisseria gonorrhoeae* infections in women by using the ligase chain reaction on patient-obtained vaginal swabs. *J. Clin. Microbiol.* 35:2129-2132.
7. Jaschek, G., C. A. Gaydos, L. E. Welsh, and T. C. Quinn. 1993. Direct detection of *Chlamydia trachomatis* in urine specimens from symptomatic and asymptomatic men by using a rapid polymerase chain reaction assay. *J. Clin. Microbiol.* 31:1209-1212.
8. Modarress, K. J., A. P. Cullen, W. J. Jaffurs, G. L. Troutman, N. Mousavi, R. A. Hubbard, S. Henderson, and A. Lorincz. 1999. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in swab specimens by the Hybrid Capture II and Pace 2 nucleic acid probe tests. *Sex. Transm. Dis.* 26:303-308.
9. Schachter, J., and W. E. Stamm. 1999. *Chlamydia*, p. 795-806. In P. R. Murray, E. J. Baron, M. A. Tenover, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.