Evaluation of the Digene Hybrid Capture II CT-ID Test for Detection of *Chlamydia trachomatis* in Endocervical Specimens

JENNIFER L. GIRDNER,[1] ALLISON P. CULLEN,[2] TERESA G. SALAMA,[3] LING HE,[2]
ATTILA LORINCZ,[2] AND THOMAS C. QUINN[1,4]*

*Division of Infectious Diseases, The Johns Hopkins University,[1] and Baltimore City Health Department,[3]
Baltimore, Digene Corporation, Silver Spring,[2] and National Institute of
Allergy and Infectious Diseases, Bethesda,[4] Maryland*

The performance characteristics of the new signal amplification-based Hybrid Capture (HC) II CT-ID test system (Digene, Silver Spring, Md.) with endocervical specimens were compared to those of tissue culture and PCR (AMPLICOR CT PCR; Roche Molecular Systems, Branchburg, N.J.) for detection of *Chlamydia trachomatis* in 587 women. HC II CT-ID identified 62 of 65 confirmed *C. trachomatis*-positive patients (sensitivity of 95.4%) and was negative for 517 of 522 patients who were negative by culture and PCR (specificity of 99.0%). Twelve of the 65 confirmed positive patients were negative by culture but were identified by both HC II CT-ID and PCR (sensitivity of culture was 81.5% [$P < 0.01$]). In comparison, PCR detected 59 of 65 positive specimens (sensitivity of 90.8%) and had a specificity of 99.6% (520 of 522). These results demonstrate that the Digene HC II CT-ID test is a highly sensitive and specific assay for the detection of *C. trachomatis* infection in endocervical specimens.

Chlamydia trachomatis infections are recognized as the most common sexually transmitted bacterial infections in the United States, with an estimated 4.5 million cases annually (8, 18). This obligate intracellular bacterium accounts for 50 to 80% of the cases of nongonococcal urethritis and cervicitis (15). Although many *C. trachomatis* infections are asymptomatic, *C. trachomatis* can result in serious long-term sequelae such as pelvic inflammatory disease, ectopic pregnancy, and infertility (16).

The traditional method for detection of *C. trachomatis* in clinical specimens has been culture. Although culture is usually sensitive and specific, it requires the recovery of live organisms and takes several days to complete, and therefore it is not efficient for a large number of specimens. In addition, factors such as specimen quality (related to collection technique), transport time, and storage of the sample can negatively influence the sensitivity of cell culture (1, 12). Other methods for diagnosis have been developed over the past few years, including direct immunofluorescence, enzyme immunoassays, and DNA probe techniques (2, 10, 13, 17, 19). Recent advances in molecular diagnostics, such as PCR (3, 4, 9), ligase chain reaction (7, 11), and transcription-mediated amplification (13), have improved the efficiency and accuracy of screening large populations by detecting small numbers of microorganisms. These tests offer higher sensitivities of detection over culture and other nonculture assays while maintaining high specificity (6, 14).

In this study, we evaluated the performance characteristics of the Hybrid Capture (HC) II CT-ID test system (Digene, Silver Spring, Md.), a nucleic acid probe-based assay in which the detection signal is amplified. The Digene HC II CT-ID test system was compared to culture and AMPLICOR CT PCR in endocervical specimens for the diagnosis of *C. trachomatis*.

Patient population. A total of 587 females between the ages of 16 and 45 attending two Baltimore City sexually transmitted disease clinics were enrolled after informed consent was ob-

tained. The study protocol was approved by the Johns Hopkins University and the Baltimore City Health Department ethical review boards. Two endocervical swabs and one Digene endocervical brush or swab were obtained from each woman enrolled in the study: one cervical swab for *C. trachomatis* culture, a cervical swab for PCR, and a cervical brush or swab for the Digene HC II CT-ID test. All enrolled women at the clinics were asked to consent to having the Digene cervical brush specimen obtained; if they did not consent to the brush, then a cervical swab specimen was obtained. For pregnant women, only cervical swabs were obtained. For nonpregnant women consenting to a brush specimen, the order of specimen collection was determined by patient identification numbers, i.e., for odd patient identification numbers, the Digene brush specimen was collected first, followed by the culture swab specimen, and visa versa for even patient identification numbers. The order of swab collection for pregnant women and women who did not consent to a brush specimen was alternated by odd and even patient identification numbers in the fashion stated above. A third cervical swab for PCR was collected after the specimens for culture and HC II CT-ID were obtained. The endocervical culture swab was placed in chlamydia transport vials containing sucrose-phosphate buffer, 10% fetal bovine serum, and antibiotics. The endocervical HC II CT-ID brush or swab was placed in 1.0 ml of specimen transport medium. The PCR swab was placed in AMPLICOR swab specimen transport medium (Roche Molecular Systems, Branchburg, N.J.). The chlamydia culture transport vials were transported at −70°C and stored at −70°C for 12 to 24 h until they were processed for culture. The HC II CT-ID swabs were transported at 2 to 4°C and stored in a −20°C freezer. If the Digene HC II CT-ID tests were to be done within 3 weeks of collection, the specimens could be held at 2 to 30°C for up to 2 weeks and at 4°C for one additional week. The PCR swabs were transported on ice and stored at 2 to 8°C until processed.

Cell culture. Chlamydia culture was performed in 96-well microtiter plates with cycloheximide-treated McCoy cell monolayers as described previously (15). After 48 h both wells were evaluated for chlamydia inclusions by immunofluorescence

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TABLE 1. Analysis of Digene HC II CT-ID and AMPLICOR CT PCR performance with endocervical specimens before and after discrepant analysis ($n = 587$)

Test and result	No. of samples with result:			
	Before discrepant analysis		After discrepant analysis[a]	
	Culture positive	Culture negative	Confirmed positive	Confirmed negative
Digene HC II CT-ID				
Positive	50	17	62	5[b]
Negative	3	517	3	517
AMPLICOR CT PCR				
Positive	47	14	59	2[b]
Negative	6	520	6	520

[a] A specimen was considered true positive in discrepant analysis if it was culture positive or positive by both Digene HC II CT-ID and Amplicor CT PCR.
[b] Specimens were also negative by DFA staining.

staining. One well was stained with monoclonal antibody to *C. trachomatis* major outer membrane protein (Microtrak Chlamydia Culture Reagent; Syva, San Jose, Calif.), and the other well was stained with antilipopolysaccharide monoclonal antibody (Sanofi Diagnostics Pasteur, Chaska, Minn.) (5).

Digene HC II CT-ID. HC II CT-ID was performed on endocervical specimens by using a new chemiluminescent, signal amplification-based solution hybridization assay. Specimens were prepared by the addition of denaturation reagent and a 45-min incubation at 65°C. Denatured specimens (DNA) were hybridized with a *C. trachomatis*-specific RNA probe cocktail for 1 h at 65°C. The RNA probes are homologous to the entire chlamydia cryptic plasmid sequence (7,500 bp) and approximately 39,000 bp of the *C. trachomatis* genome (4%). There are approximately 10 copies of the cryptic plasmid sequence per organism and a single copy of the remaining genomic sequences per organism.

The hybridization mixture was transferred to a microplate coated with antibodies specific for RNA-DNA hybrids and shaken for 60 min at room temperature to capture the hybrids. The microplate was decanted and reacted with alkaline phosphatase-conjugated antibodies specific for RNA-DNA hybrids for 30 min at room temperature. The capture microplate was decanted and washed six times to remove any unbound antibody conjugate. The signal is generated by the cleavage of a chemiluminescent substrate. Substantial signal amplification is achieved because several alkaline phosphatase molecules are conjugated to each antibody and multiple antibodies bind to each captured RNA-DNA hybrid. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted and measured as relative light units with a luminometer. The cutoff value for the test is the mean number of relative light units RLU of three replicates of the positive control which is tested in each assay. The positive control is 1 pg of cloned cryptic plasmid DNA per ml in Digene specimen transport medium. The limit of detection for the HC II CT-ID test ranges from 50 to 2,500 elementary bodies/assay for the 15 *C. trachomatis* serovars.

PCR. Roche AMPLICOR CT PCR was performed in 96-microwell plates according to the manufacturer's instructions and as described previously (9).

Discrepant analysis. Specimens that were culture negative but positive by HC II CT-ID and/or PCR were also analyzed by direct fluorescent-antibody (DFA) staining of sedimented culture transport media for elementary bodies (2). A patient was considered true positive for *C. trachomatis* if the culture was

positive or if at least two of the following tests were positive: HC II CT-ID, PCR, or DFA staining.

Of the 587 females, 65 (11.1%) were considered true positives for *C. trachomatis* (Table 1). Among these 65 positive females, 62 (10.6% of the total) were HC II CT-ID positive and 3 were HC II CT-ID negative but culture positive, yielding a sensitivity for HC II CT-ID of 95.4% (Table 2). An additional five specimens were HC II CT-ID positive, culture and PCR negative, and confirmed negative by DFA staining (HC II CT-ID specificity of 99.0%) (Table 2). Fifty-nine specimens were positive for PCR (Table 1). Of the remaining six true-positive specimens, two were culture positive only and four were also HC II CT-ID positive, thus yielding a sensitivity of 90.8% for AMPLICOR CT PCR (Table 2). Two specimens were PCR positive but culture and HC II CT-ID negative; these were also negative by DFA staining (AMPLICOR specificity of 99.6%) (Table 2). Fifty-three of the sixty-five true-positive females were positive by culture. Of the 12 HC II CT-ID- and PCR-positive, culture-negative specimens, only 2 were positive by DFA staining. The sensitivity of *C. trachomatis* culture was therefore 81.5% (Table 2), which was significantly lower than the sensitivities of HC II CT-ID and PCR ($P < 0.01$, McNemar's chi-square statistic). The specificity of culture was 100%, by definition.

One hundred twenty-two specimens from the study were rerun on two separate occasions by using the HC II CT-ID assay to test reproducibility. The agreement between the two runs was 98.4%. The two runs gave highly reproducible results, with a kappa value of 0.948 and an r^2 value of 0.96.

This paper describes the evaluation of the Digene HC II CT-ID signal amplification-based test for the detection of *C. trachomatis* from endocervical specimens. HC II CT-ID was highly sensitive and specific for the detection of *C. trachomatis* infection compared to culture and was comparable to a nucleic acid amplification-based method, PCR, known to be a highly sensitive technique for detecting *C. trachomatis* DNA in endocervical swabs (14).

The difference in the sensitivity of HC II CT-ID and AMPLICOR CT PCR with endocervical specimens was not statistically significant and could be a result of several factors. Different clinicians use different collection techniques. Reliable results are dependent on consistent specimen collection techniques; however, it is impossible to guarantee that there was no variability in the specified collection techniques for this or any other assay. In addition, the sensitivity of PCR could have been affected since it was always performed on the third cervical swab. Furthermore, obtaining a positive culture and a negative HC II CT-ID or AMPLICOR CT PCR result may also reflect variations in specimen collection or the presence of inhibitors which may cause false-negative results. For clinical purposes, retesting of all HC II CT-ID specimens to assess the presence of such possible inhibitors is not practical and therefore was not performed. However, such an evaluation would be

TABLE 2. Resolved performance characteristics with endocervical specimens for detection of *C. trachomatis* by Digene HC II CT-ID and AMPLICOR CT PCR compared to culture ($n = 587$)[a]

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Digene HC II CT-ID	95.4	99.0	92.5	99.4
AMPLICOR CT PCR	90.8	99.6	96.7	98.9
Culture	81.5	100.0	100.0	97.8

[a] PPV, positive predictive value; NPV, negative predictive value.

an important step in determining the true reliability of an amplification assay.

The HC II CT-ID assay is well adapted for high-throughput screening, since 90 clinical specimens per microplate can be processed in 5 h. When only one specimen is used, however, the Digene HC II CT/GC test algorithm is an excellent test for screening people for the most common sexually transmitted diseases. For example, if the initial test for *C. trachomatis* and gonorrhea is negative, no further testing is required, and a cost savings results for low-prevalence populations. This more accurate screening and identification method has an immediate application in asymptomatic populations, where it may lead to a reduction in the spread of infection and in the risk for complications and sequelae.

Our results demonstrate that HC II CT-ID is more sensitive than culture in identifying chlamydial organisms in endocervical specimens. The assay is comparable to other currently available nucleic acid target amplification-based tests, such as PCR. In addition, the HC II CT-ID test provides a rapid and reproducible assay that is easy to use and efficient for the detection of *C. trachomatis*.

REFERENCES

1. **Aarnaes, S. L., E. M. Peterson, and I. M. de la Maza.** 1984. The effect of media and temperature on the storage of *Chlamydia trachomatis*. Am. J. Clin. Pathol. **81:**237–239.
2. **Barnes, R. C.** 1989. Laboratory diagnosis of human chlamydial infections. Clin. Microbiol. Rev. **2:**119–136.
3. **Bauwens, J. E., A. M. Clark, and W. E. Stamm.** 1993. Diagnosis of *Chlamydia trachomatis* endocervical infections by a commercial polymerase chain reaction assay. J. Clin. Microbiol. **31:**3023–3027.
4. **Bianchi, A., C. Scieux, N. Brunat, D. Vexiau, M. Kermanach, P. Pezin, M. Janier, P. Morel, and P. H. Langrange.** 1994. An evaluation of the polymerase chain reaction AMPLICOR *Chlamydia trachomatis* in male urine and female urogenital specimens. Sex. Transm. Dis. **21:**196–200.
5. **Boman, J., C. Gaydos, P. Juto, G. Wadell, and T. C. Quinn.** 1997. Failure to detect *Chlamydia trachomatis* in cell culture by using a monoclonal antibody directed against the major outer membrane protein. J. Clin. Microbiol. **35:** 2679–2680.
6. **Burczak, J. D., M. A. Chernesky, S. J. Tomazic-Allen, T. C. Quinn, J. Carrino, J. Schachter, W. E. Stamm, and H. H. Lee.** 1994. Application of ligase chain reaction to the detection of *Chlamydia trachomatis* in urogenital specimens from men and women, p. 322–329. *In* J. Orfila, G. I. Byrne, and M. A. Chernesky, Chlamydial infections. Societa Editrice Esculapio, Bologna, Italy.
7. **Chernesky, M. A., D. Jang, H. Lee, J. D. Burczak, H. Hu, J. Sellors, S. J. Tomazic-Allen, and J. B. Mahony.** 1994. Diagnosis of *Chlamydia trachomatis* infections in men and women by testing first-void urine by ligase chain reaction. J. Clin. Microbiol. **32:**2682–2685.
8. **Institute of Medicine.** 1997. The hidden epidemic—confronting sexually transmitted diseases, p. 28–68. National Academy Press, Washington, D.C.
9. **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.
10. **Kellogg, J. A.** 1989. Clinical and laboratory considerations of culture vs. antigen assays for detection of *Chlamydia trachomatis* from genital specimens. Arch. Pathol. Lab. Med. **113:**453–460.
11. **Lee, H., M. Chernesky, J. Schacter, J. Burczak, W. Andrews, S. Muldoon, G. Leckie, and W. Stamm.** 1995. Diagnosis of *Chlamydia trachomatis* genitourinary infection in women by ligase chain reaction assay of urine. Lancet **345:** 213–216.
12. **Mahony, J. B., and M. A. Chernesky.** 1985. Effect of swab type and storage temperature on the isolation of *Chlamydia trachomatis* from clinical specimens. J. Clin. Microbiol. **22:**865–867.
13. **Pasternack, R., P. Vuorinen, and A. Miettinen.** 1997. Evaluation of the Gen-Probe *Chlamydia trachomatis* transcription-mediated amplification assay with urine specimens from women. J. Clin. Microbiol. **35:**676–678.
14. **Quinn, T. C.** 1994. Recent advances in diagnosis of sexually transmitted diseases. Sex. Transm. Dis. **21:**19–27.
15. **Stamm, W. E.** 1988. Diagnosis of *Chlamydia trachomatis* genitourinary infections. Ann. Intern. Med. **108:**710–717.
16. **Stamm, W. E., and K. K. Holmes.** 1990. *Chlamydia trachomatis* infections of the adult, p. 181–193. *In* K. K. Holmes, P. A. Mardh, P. F. Sparling, and P. J. Wiesner (ed.), Sexually transmitted diseases. McGraw-Hill Co., New York, N.Y.
17. **Taylor-Robinson, D., and B. J. Thomas.** 1991. Laboratory techniques for the diagnosis of chlamydial infections. Genitourin. Med. **67:**256–266.
18. **Washington, A., R. Johnson, and L. Sanders.** 1987. *Chlamydia trachomatis* infections in the United States: what are they costing us? JAMA **257:**2070–2072.
19. **Wu, C. H., M. F. Lee, S. C. Yin, D. M. Yand, and S. F. Cheng.** 1992. Comparison of polymerase chain reaction, monoclonal antibody based enzyme immunoassay, and cell culture for detection of *Chlamydia trachomatis* in genital specimens. Sex. Transm. Dis. **19:**193–197.