JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2002, p. 281–283 Vol. 40, No. 1
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.1.281–283.2002

Evaluation of a Strand Displacement Amplification Assay (BD ProbeTec-SDA) for Detection of *Neisseria gonorrhoeae* in Urine Specimens

Deniz Akduman,[1] Josephine M. Ehret,[1,2] Kimberly Messina,[1] Sharron Ragsdale,[1] and Franklyn N. Judson[1,2,3]*

Denver Public Health Department[1] and Departments of Medicine[2] and Preventive Medicine,[3] University of Colorado Health Sciences Center, Denver, Colorado

Received 13 June 2001/Returned for modification 25 July 2001/Accepted 15 October 2001

The performance of a strand displacement amplification assay (the BDProbeTec-SDA assay) in detecting *Neisseria gonorrhoeae* in urine specimens was evaluated. When performed under stringent quality control conditions, the BDProbeTec-SDA assay is a sensitive, specific, and efficient method for the screening of large numbers of noninvasively obtained specimens. Because the predictive value of an assay is a function of the prevalence of the disease, culture confirmation is needed for samples with positive results from populations in which the prevalence of a disease is low or in situations in which false-positive results may have important medical, psychosocial, or medicolegal consequences.

Genitourinary tract infections due to *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are major causes of morbidity in sexually active individuals (7). Approximately 3 million new cases of *C. trachomatis* infection and 650,000 new cases of *N. gonorrhoeae* infection occur annually in the United States

Asymptomatic infections in both men and women, along with coinfections and overlapping symptoms, can make specific clinical diagnosis difficult. Failure to provide effective treatment can lead to epididymitis in men and pelvic inflammatory disease and its sequelae of infertility, ectopic pregnancy, and chronic pelvic pain in women (12). There also is some evidence that gonococcal infections may facilitate the transmission of human immunodeficiency virus (6). As a result, prevention and control efforts increasingly are being focused on early diagnosis and treatment, especially in high-risk adolescents and young adults ≤24 years of age.

The reference standard for detection of *N. gonorrhoeae* has been isolation by culture (1, 5), which has a high specificity and which can be used for susceptibility testing (1). Disadvantages include stringent transport and storage requirements and a bacterial growth time of 2 days or more. In addition, detection by culture usually involves an invasive procedure: a pelvic examination for women and insertion of an urethral swab for men.

For a variety of specimens to be tested for *N. gonorrhoeae* or *C. trachomatis* including urethral and endocervical swab and urine specimens, nucleic acid detection assays such as PCR, ligase chain reaction, and transcription-mediated amplification possess higher sensitivities than older methods (2–4, 8–11, 13). However, each of these tests has limitations including variable sensitivities to inhibitors; limited throughput; high labor costs; and requirements for separate sample preparation, amplification, and detection areas and dedicated equipment. Thus, there is a need for a more user-friendly, sensitive, and specific diagnostic system which can test for *C. trachomatis* and *N. gonorrhoeae* simultaneously in large numbers of noninvasively obtained specimens.

The BDProbeTec-SDA system (Becton Dickinson Microbiology Systems, Sparks, Md.) is a new semiautomated system which uses strand displacement amplification for the simultaneous detection of *C. trachomatis* and *N. gonorrhoeae*. Products are detected in solution with fluorescent detector probes. Positive and negative controls for specimen processing are included in the kit along with an amplification control to monitor assay inhibition.

We evaluated the diagnostic performance of the BDProbeTec-SDA assay for the detection of *N. gonorrhoeae* infection by comparing the results with those obtained by culture and by retesting all specimens with positive results and 280 specimens with negative results. The performance of the system for the detection of *C. trachomatis* was not evaluated.

A total of 3,544 urine specimens were collected between 17 October 2000 and 11 January 2001 from patients attending the Denver Metro Health (sexually transmitted disease [STD]) Clinic. Matched endocervical swab specimens from women and urethral swab specimens from men were cultured on modified Thayer-Martin and chocolate agar biplates. Specimen processing and BDProbeTec-SDA assays were performed by two experienced technicians according to the manufacturer's instructions (package insert, revised March 2000; Becton Dickinson Microbiology Systems). Amplification controls were used in each assay to monitor inhibition. The BDProbeTec-SDA assay was repeated twice with 152 initially positive urine specimens (144 specimens from men, 8 specimens from women) and 280 initially negative urine specimens (229 specimens from men, 51 specimens from women). Specimens with a method other than acceleration (MOTA) reading of ≥2,000 and an amplification control reading of ≥1,000 were recorded as positive for *N. gonorrhoeae*; specimens with a MOTA value of <2,000 and an amplification control MOTA value of ≥1,000

* Corresponding author. Mailing address: Denver Public Health, 605 Bannock St., Denver, CO 80204-4507. Phone: (303) 436-7200. Fax: (303) 436-7211. E-mail: Frank.Judson@dhha.org.

TABLE 1. Performance of BDProbeTec-SDA system compared with that of culture for detection of *N. gonorrhoeae* in 3,544 urine specimens from patients attending the Denver Metro Health (STD) Clinic[a]

Test result	No. of specimens (% of total)
BDProbeTec-SDA assay positive, culture positive	129 (3.64)
BDProbeTec-SDA assay culture negative	23 (0.65)
BDProbeTec-SDA assay negative, culture positive	1 (0.03)
BDProbeTec-SDA assay negative, culture negative	3,391 (95.68)

[a] The performance of the BDProbeTec-SDA assay was based on a prevalence by culture of 3.63%.

were recorded as negative for *N. gonorrhoeae*. Specimens with an amplification control MOTA value of <1,000 were recorded as indeterminate, as described in the package insert.

N. gonorrhoeae was detected in 129 specimens by culture and in 152 specimens by the BDProbeTec-SDA assay (Table 1). On the basis of a prevalence of 3.63% by culture, the sensitivity, specificity, positive predictive value, and negative predictive value for the BDProbeTec-SDA system were 99.2, 99.3, 84.9, and 99.9%, respectively. The overall discrepancy rate was 24 of 3,544 specimens (0.7%). Upon retesting, 128 of 129 (99.2%) of the specimens with BDProbeTec-SDA assay-positive, culture-positive results remained BDProbeTec-SDA assay positive, whereas 7 of 23 (30.4%) of the specimens with BDProbeTec-SDA assay-positive, culture-negative results were BDProbeTec-SDA assay positive. Fifty percent of the specimens with discrepant positive results had MOTA values in the range of 2,000 to 10,000. The rates of discrepancy of the results between the BDProbeTec-SDA assay and culture did not differ significantly between the two technicians ($P = 0.09$). Of 280 specimens initially BDProbeTec-SDA assay and culture negative, 8 (2.9%) were positive for *N. gonorrhoeae* by one of two repeat tests. MOTA values ranged between 2,267 and 20,334.

Our results suggest that the BDProbeTec-SDA system, which is based on real-time fluorescence detection and strand displacement amplification, provides a sensitive, specific, and reproducible method for the detection of *N. gonorrhoeae*. The assay system takes 1.5 to 2 h to perform up to 96 tests. Amplification and detection steps are performed automatically within a closed instrument, limiting contamination and allowing operation in a single room. An amplification control monitors each specimen for the presence of inhibitors and reduces the number of false-negative results. A negative test virtually rules out culture-positive *N. gonorrhoeae*.

To maintain high sensitivity and specificity, tests should be performed under stringent quality control conditions and laboratorians must be aware of the potential for sample-to-sample and environmental contamination. Problems originating from the sample processing area and splatter during pipetting (especially when transferring specimens from priming wells to amplification wells) may have caused some of our BDProbeTec-SDA assay-positive, culture-negative discrepant results. Gloves should be changed frequently, especially between the processing and the amplification areas.

Recently, Becton Dickinson has recommended the use of a hand drill for removal of caps. The drill is used after specimens have been lysed in the heat block. Due to heating, liquid is frequently present around the tops of the tubes, and it is difficult to remove caps that are placed closely in the block without cross-contamination. The company now recommends that the block be tapped on the counter to shake down fluid before removal of the caps.

The work area and the automatic pipettor should be cleaned frequently with 20% sodium hypochlorite-detergent solution. To improve reproducibility, work surfaces are monitored weekly by performing the BDProbeTec-SDA assay with environmental samples taken before and after routine cleaning. If the samples test positive, work surfaces are recleaned and retested on the following day. The performance of the assay should be monitored continuously, and training should be updated.

Another explanation for discrepant results is the higher sensitivity of the BDProbeTec-SDA system, which may detect nucleic acid from as few as 10 gonococci. Becton Dickinson informed us that BDProbeTec-SDA assay-positive specimens with MOTA scores above 10,000 are true positives. Therefore, we retest specimens with MOTA values in the range of 2,000 to 10,000, which represent about 50% of specimens with discrepant results.

The benefits of nucleic acid amplification tests are more apparent in populations with a high prevalence of STDs, such as STD clinic patients and younger men and women with new or multiple sexual partners. Because the positive predictive value is a function of prevalence, culture confirmation is needed for samples with positive results from populations in which the prevalence of STD, is low, for which false-positive results can negatively affect low-risk sexual relationships (e.g., long-term, mutually monogamous relationships) as well as care provider-patient relationships. Even in our laboratory, which serves an STD clinic population with an overall *N. gonorrhoeae* prevalence rate of 4.3%, we repeat the assay in duplicate for all specimens that are initially positive. Specimens with positive results upon retesting are reported as having positive results.

Although culture remains the only test that can definitively identify *N. gonorrhoeae* for medicolegal purposes and is essential for susceptibility testing, the BDProbeTec-SDA system offers a sensitive, specific, and efficient method for the screening of a large number of noninvasively obtained specimens for *N. gonorrhoeae*.

REFERENCES

1. **Baron, E. J., G. H. Cassel, L. B. Duffy, D. A. Eschenbach, J. R. Greenwood, S. M. Harvey, N. E. Madinger, E. M. Peterson, and K. B. Waites.** 1993. Cumitech 17a, Laboratory diagnosis of female genital tract infections. Coordinating ed., E. J. Baron. American Society for Microbiology, Washington, D.C.
2. **Bassiri, M., H. Y. Hu, M. A. Domeika, J. Burczak, L. O. Svensson, H. H. Lee, and P. A. Mardh.** 1995. Detection of *Chlamydia trachomatis* in urine specimens from women by ligase chain reaction. J. Clin. Microbiol. **33:**898–900.
3. **Buimer, M., G. J. J. Doornum, S. Ching, P. G. Peerbooms, P. K. Plier, D. Ram, and H. H. Lee.** 1996. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by ligase chain reaction-based assays with clinical specimens from various sites: implications for diagnostic testing and screening. J. Clin. Microbiol. **34:**2395–2400.
4. **Catry, M. A., M. J. Borrego, J. Cardoso, J. Azevedo, and I. Santo.** 1995. Comparison of the Amplicor *Chlamydia trachomatis* test and cell culture for the detection of urogenital chlamydial infections. Genitourin. Med. **71:**247–250.
5. **Chapin-Robertson, K.** 1993. Use of molecular diagnostics in sexually transmitted diseases. Diagn. Microbiol. Infect. Dis. **16:**173–184.
6. **Cohen, M. S., I. F. Hoffman, R. A. Royce, P. Kazembe, J. R. Dyer, C. C. Daly, D. Zimba, P. L. Vernazza, M. Maida, S. A. Fiscus, and J. J. Erron, Jr.** 1997. Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. Lancet **349:** 1868–1873.
7. **De Schryver, A., and A. Meheus.** 1990. Epidemiology of sexually transmitted

diseases: the global picture. Bull. W. H. O. **68:**639–654.
8. **Domeika, M., M. Bassiri, and P. A. Mardh.** 1994. Diagnosis of genital *Chlamydia trachomatis* infections in asymptomatic males by testing urine by PCR. J. Clin. Microbiol. **32:**2350–2352.
9. **Martin, D. H., C. Cammarata, B. Van Der Pol, R. B. Jones, T. C. Quinn, C. A. Gaydos, K. Crotchfelt, J. Schachter, J. Moncada, D. Junkind, B. Turner, and C. Peyton.** 2000. Multicenter evaluation of Amplicor and automated Cobas Amplicor CT/NG tests for *Neisseria gonorrhoeae*. J. Clin. Microbiol. **38:**3544–3549.
10. **Pasternak, R., P. Vuorinen, and A. Miettinen.** 1997. Evaluation of the GenProbe *Chlamydia trachomatis* transcription-mediated amplification assay with urine specimens from women. J. Clin. Microbiol. **35:**676–678.
11. **Pasternak, R., P. Vuorinen, T. Pitkajarvi, M. Koskela, and A. Miettinen.** 1997. Comparison of manual Amplicor PCR, Cobas Amplicor PCR, and LCx assays for detection of *Chlamydia trachomatis* infection in women by using urine specimens. J. Clin. Microbiol. **35:**402–405.
12. **Walker, C. K., J. G. Kahn, A. E. Washington, H. B. Peterson, and R. L. Sweet.** 1993. Pelvic inflammatory disease: metaanalysis of antimicrobial regimen efficacy. J. Infect. Dis. **168:**969–978.
13. **Xu, K., V. Glanton, S. R. Johnson, C. Beck-Sauge, V. Bhullar, D. H. Candal, K. S. Pettus, C. E. Farshy, and C. M. Black.** 1998. Detection of *Neisseria gonorrhoeae* infection by ligase chain reaction testing of urine among adolescent women with and without *Chlamydia trachomatis* infection. Sex. Transm. Dis. **25:**533–538.