

Multicenter Evaluation of the BDProbeTec™ ET System in Detecting *Chlamydia trachomatis* and *Neisseria gonorrhoeae* from Endocervical and Urine Specimens in Women

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ABSTRACT (Revised)

■ The performance of the BDProbeTec™ ET System (BDPT) for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) was evaluated. BDPT uses thermophilic Strand Displacement Amplification (SDA) technology to simultaneously amplify and detect target DNA.

Endocervical swab and first catch urine (FCU) specimens were obtained from 562 female patients attending family planning clinics. BDPT performance was compared to culture and an “enhanced” gold standard based upon the combined criteria of culture, direct fluorescent antibody testing, and another nucleic acid amplification test (LCR).

The prevalence of CT was 5.0% and for GC, 1.5%. The sensitivity of the CT assay compared to the “enhanced” gold standard for endocervical swab specimens was 96.4% (27/28), with a specificity of 98.5% (515/523); and for FCU specimens the sensitivity was 100.0% (26/26), with a specificity of 98.2% (427/435). The sensitivity of the GC assay compared to the “enhanced” gold standard for endocervical swab specimens was 100% (8/8), with a specificity of 99.4% (536/539); and for FCU specimens the sensitivity was 83.3% (5/6), with a specificity of 99.3% (441/444). CT culture sensitivity was 64.3% (18/28) and for GC culture, 87.5% (7/8).

The BDProbeTec™ ET System is a highly sensitive and specific DNA amplification assay.

INTRODUCTION

The most prevalent sexually transmitted disease in the United States today is caused by *Chlamydia trachomatis* and the number of reported cases continues to rise.⁽¹⁾ During 1998 the nation has experienced the first increase in the number of reported cases of gonorrhea following a several year decline in reported cases. The significance of serious complications related to chlamydia and gonorrhea infections has been well established.⁽²⁾ Urethritis, epididymitis, proctitis, cervicitis, pelvic inflammatory disease, infant pneumonia, and conjunctivitis are some of the potential outcomes of infection with these diseases. The advent of managed care and shrinking public health budgets have made rapid early accurate diagnosis and adequate treatment even more critical. The capability of testing using non-invasive specimens such as urine and the ability to acquire specimens in non-traditional venues has been reported as an important intervention effort.^(3,4)

Traditional cell culture methods have been the gold standard for diagnosis of chlamydia infections. However, cell culture methods are expensive, time consuming and subject to lab-to-lab variation. The introduction of enzyme immunoassay (EIA) tests and the DNA probe test (Gen-Probe, Inc.) for direct detection of antigen in patient samples provided an alternative to tissue culture.⁽⁵⁾ The sensitivity and specificity of EIA and DNA probe tests are comparable to culture. Recently, nucleic acid amplification methods based on the polymerase chain reaction (PCR), ligase chain reaction (LCR) and transcription mediated amplification (TMA) assays have been reported to offer improved performance over culture and other non-culture methods.^(6,7,8) These tests have expanded the capability for obtaining non invasive specimens in non-traditional settings. The latest amplified procedure to be evaluated in the United States is the Becton Dickinson BDProbeTec™ ET (BDPT) assay.⁽⁹⁾

The BDPT assay utilizes strand displacement amplification (SDA) to simultaneously amplify and detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* deoxyribonucleic acid (DNA) in male urethral swab and urine specimens and female endocervical and urine specimens.

The purpose of this study was to assess the performance characteristics of the BDPT assay with female endocervical swab and first catch urine (FCU) specimens in a low prevalence population. The BDPT assay performance was compared to a gold standard, which compared BDPT to standard culture methods and DFA for CT and culture for GC. The BDPT assay performance was further compared to an “enhanced” gold standard defined as a laboratory diagnosis of infection based upon the combined criteria of culture, direct fluorescent antibody testing of the spun down portion of the CT transport media, GC culture confirmation, and another nucleic acid amplification test (LCR).

METHODS

Four endocervical swabs and a urine specimen were collected from each female patient seen in family planning clinics or OB/GYN clinics. The swab order of collection was randomized. One swab sample was used for the culture of *C. trachomatis*, one swab sample was used for the culture of *N. gonorrhoeae*, one swab sample was tested by the BDPT assay and the fourth

swab sample was tested by the LCR assay (Abbott Laboratories). The patient should not have urinated within one hour. Urine specimens were split into two aliquots. The aliquot for the BDPT test was stabilized by the addition of a urine processing pouch (UPP) containing resins. Urine specimens were tested by the BDPT and LCR assays. All swab and urine specimens were transported and stored at the laboratory according to the manufacturer's instructions. The manufacturer's procedure for LCR (Abbott Laboratories) was followed for swab and urine specimen processing and amplification. Each laboratory was required to pass proficiency for the BDProbeTec ET system as well as CT tissue culture and GC culture prior to beginning the study.

Swab specimens to be processed in the BDPT assay are added to a diluent tube, mixed for 5-10 seconds, expressed and then vortexed for 5-10 seconds. Urine specimens to be processed in the BDPT assay were aliquoted to 4 ml., centrifuged at 2000 x g, and decanted. The pellet was resuspended in 2 ml. of diluent. After processing, the processed swab and urine specimens are handled in the same manner throughout the assay. The processed sample tubes are placed in the lysing rack, incubated at 110° C. for 30 minutes, cooled for 15 minutes. The specimens are pipetted into priming microwells and incubated at least 20 minutes (but up to six hours) at room temperature. The priming and amplification plates are then transferred to a priming and warming heater for 10

minutes. After 10 minutes, the samples are transferred from the priming microwells into the amplification microwells and immediately sealed. The amplification microwells are placed in the BDProbeTec ET instrument where simultaneous amplification and detection occurs for 60 minutes. Refer to Figures 1 and 2.

M4 transport media (MicroTest, Inc.) was used for CT culture. The transport media was vortexed and the swab was removed CT culture. A portion of the transport media was inoculated onto McCoy shell vials, centrifuged, incubated, and stained with fluorescent antibody stain per manufacturer's instructions.

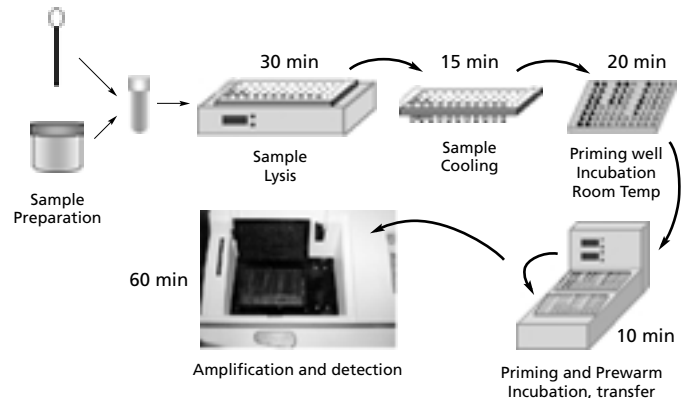
Directly inoculated Modified Thayer Martin (MTM) or Martin-Lewis culture plates were immediately put into candle jars or transport bags and incubated until transferred to the laboratory periodically throughout the day. At the laboratory, the MTM was cross-streaked and then incubated for approximately 48 hours. GC cultures were screened using the oxidase reaction and Gram stain. Positive cultures were confirmed using two methods one of which was biochemical.

Discrepant specimens (specimens which were culture negative and amplified method positive) were further analyzed by direct fluorescent antibody (DFA) testing of the CT transport media per manufacturer's instructions. The culture swabs and urine specimens were stored at -70°C until all testing was completed.

Figure 1: BDProbeTec ET System



Figure 2: BDProbeTec ET System Workflow



RESULTS

A total of 562 patients were enrolled after obtaining informed consent. All patients enrolled had a swab specimen and most of the specimens had paired urine specimens. In most cases both the CT and GC assays were performed, except where one of the assays was non-compliant. The prevalence of CT or GC infection based on the enhanced gold standard was 5.0% and 1.5%, respectively. For CT, a patient was considered positive by the enhanced gold definition if: CT culture or DFA was positive, or if LCR and DFA were positive or if LCR was positive in both swab and urine specimens. For GC, a patient was considered positive

if: GC culture was positive or if LCR was positive in both swab and urine specimens. By expanding the definition, an additional 8 patients with *C. trachomatis* infections and 1 patient with *N. gonorrhoeae* were identified. CT tissue culture sensitivity was 64.3% (18/28). GC culture sensitivity was 87.5% (7/8). Indeterminate rates for urine specimens were 7.2 and 7.6% for CT and GC, respectively. The sensitivity and specificity based upon the gold standard and the "enhanced" gold standard are summarized in Table 1. The BDPT assay sensitivity and specificity compared to the LCR assay is summarized in Table 2.

RESULTS

Table 1

| SENSITIVITY AND SPECIFICITY FOR BDPT COMPARED TO GOLD STANDARD AND ENHANCED GOLD STANDARD TESTING | | | | | | | | | |
|---|-------------|--|-------|-------------|-------|---|-------|-------------|-------|
| Assay | Sample type | Gold Standard (vs. Culture & DFA for CT and culture for GC) | | | | Enhanced Gold Standard (Infected Patient Status) | | | |
| | | Sensitivity | | Specificity | | Sensitivity | | Specificity | |
| | | No. | & (%) | No. | & (%) | No. | & (%) | No. | & (%) |
| CT | Swab | 20/20 | 100% | 516/531 | 97.2% | 27/28 | 96.4% | 515/523 | 98.5% |
| | FCU | 19/19 | 100% | 427/442 | 96.6% | 26/26 | 100% | 427/435 | 98.2% |
| GC | Swab | 7/7 | 100% | 536/540 | 99.3% | 8/8 | 100% | 536/539 | 99.4% |
| | FCU | 4/5 | 80.0% | 441/445 | 99.1% | 5/6 | 83.3% | 441/444 | 99.3% |

Table 2

| SENSITIVITY AND SPECIFICITY VS. GOLD STANDARD RESULTS FOR BDPT COMPARED TO LCR | | | | | | | | | |
|--|-------------|-------------|-------|-------------|-------|-------------|-------|-------------|-------|
| Assay | Sample Type | BDPT | | | | LCR | | | |
| | | Sensitivity | | Specificity | | Sensitivity | | Specificity | |
| | | No. | & (%) | No. | & (%) | No. | & (%) | No. | & (%) |
| CT | Swab | 20/20 | 100% | 516/531 | 97.2% | 20/20 | 100% | 530/542 | 97.8% |
| | FCU | 19/19 | 100% | 427/442 | 96.6% | 18/18 | 100% | 467/479 | 97.7% |
| GC | Swab | 7/7 | 100% | 536/540 | 99.3% | 5/7 | 71.4% | 541/543 | 99.6% |
| | FCU | 4/5 | 80.0% | 441/445 | 99.1% | 3/5 | 60.0% | 478/482 | 99.2% |

CONCLUSION

The BDProbeTec™ ET System is a highly sensitive and specific DNA amplification assay for the detection of chlamydia and gonorrhea infections in women for both endocervical and urine specimens. The sensitivity and specificity compared with the enhanced gold standard for female genital swab specimens was 96.4% and 98.5% for CT and 100% and 99.4% for GC, respectively. The sensitivity and specificity for female urine specimens was 100% and 98.2% for CT and 83.3% and 99.3% for GC, respectively. Ten (36%) more cases of chlamydia infection were identified using BDPT compared to culture alone. Utilizing a noninvasive specimen such as urine has advantages if the patient is reluctant to allow a swab sample to be collected or if the collection venue does not permit genital swab sampling.

The BDPT assay can be performed in a matter of hours, thus eliminating delays experienced with non-amplification and other amplification assays. The throughput allows for up to six runs per day to be performed with the assay. Each run can be configured to run 32 specimens for detection of CT and GC with the amplification control for each specimen. The BDPT assay does not require specialized equipment such as a thermalcycler or separate rooms for workflow.

Swab and urine specimens were held at 2-30°C for up to four days after collection for the protocol. These broad storage and transport conditions allow more flexibility in the use of BDPT assay as compared to culture, other non-culture and other amplified procedures, which require more stringent specimen handling. Simultaneous amplification and detection under sealed conditions eliminates the potential for contamination during amplification. The use of an amplification control reduces the possibility of reporting false negative results due to inhibition of amplification. The performance of the BDPT when compared to Abbott LCR was comparable.

In conclusion, the Becton Dickinson BDProbeTec™ ET assay performs extremely well when compared to tissue culture or LCR or other amplification procedures. The assay possesses endocervical swab performance characteristics that exceed those pub-

lished for tissue culture comparisons to EIA and nucleic acid probe assays. The BDPT assay has swab and urine specimen performance characteristics that are comparable to or exceed those published for tissue culture comparisons with other amplified assays used in STD diagnosis such as PCR, LCR or TMA.

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