

Evaluation of the BDProbeTec™ ET System for *N. gonorrhoeae* and *C. trachomatis* Diagnosis Using Urine or Swab Specimens from Females Attending STD Clinics

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ABSTRACT

■ **Background:** Infections due to *Neisseria gonorrhoeae* (GC) and *Chlamydia trachomatis* (CT) are the two most common reportable infections in the United States. Non-culture tests for these pathogens have many advantages for clinicians.

Objective: To evaluate the performance of a new, molecular-based simultaneous amplification and detection system employing Strand Displacement Amplification™ for GC and CT diagnosis. The BDProbeTec™ ET, (BDMS Sparks, MD) uses both swab and urine specimens.

Methods. Urine and swab specimens from males and females attending an STD clinic were evaluated in comparison to culture for each pathogen. Discrepancies between BDProbeTec™ ET and culture results were resolved using Abbott LCR testing for both pathogens and DFA analysis for chlamydia.

Results. Data are currently available for the first 72 of 150 patients to be enrolled in this pilot study. The BDProbeTec™ ET sensitivity using both swab and urine specimens was 100% for gonorrhea diagnosis with a specificity of 99.1%. For CT, the BDProbeTec™ ET assay detected more infections than standard cell culture (16 for BDProbeTec™ ET vs 12 for culture) and yielded a resolved (using DFA and LCR testing) sensitivity of 94.1% (specificity 96.7%). Data will be presented for the entire study.

Conclusions: The BDProbeTec™ ET Assay for diagnosis for GC and CT infection is a promising method for STD diagnosis using either urine or genital swab specimens.

INTRODUCTION

BACKGROUND. The most common reportable sexually transmitted infections reported in the United States are caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Both infections may be asymptomatic, yet can be transmitted to others and, in women, may advance to complications such as pelvic inflammatory disease, ectopic pregnancy, and infertility. As a result, screening of asymptomatic, at risk persons is an important element of STD control efforts. Development of DNA amplification techniques more sensitive than culture have introduced methods of specimen collection which may be more acceptable to patients for use as screening methods.

OBJECTIVE/OVERVIEW. This clinical trial evaluation examined the performance of the BDProbeTec ET System for detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* which utilizes strand displacement amplification (SDA) technology. This test utilizes isothermal DNA amplification technology. The BDProbeTec ET System is based on simultaneous amplification and detection of target DNA by the use of amplification primers in the presence of a fluorescent-labeled detector probe in disposable microwell-strips. In this clinical evaluation, the performance of the BDProbeTec ET System for detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in voided urine and swab specimens was compared to standard culture methods, DFA & Abbott LCR.

METHODS

SPECIMEN COLLECTION: Asymptomatic and symptomatic females attending STD Clinics were asked to participate in the study. Four endocervical swab specimens from females were collected. Following use of the first swab for *N. gonorrhoeae* culture, the order of swabs for the BDProbeTec ET System, *C. trachomatis* culture, and Abbott LCx⁷ was randomized.

Before pelvic examination, each female was asked to provide a urine specimen for testing. Four mls of urine were immediately aliquoted for LCx analysis, then a urine processing pouch was added to the remaining urine for testing using the BDProbeTec ET System.

DETAILED DESCRIPTION OF THE BDPROBETEC ET ASSAY (REFER TO COLOR CHART)

Swabs for testing by the BDProbeTec ET method were processed by inserting the swab into a prefilled labeled sample diluent tube and mixed by swirling for 5-10 seconds. Swabs were then expressed on the inside of the tube, back into the bottom of the tube. Specimens were tightly recapped and vortexed for 5 seconds.

2. Urine specimens were mixed by swirling and 4 mls were immediately pipetted into the appropriately labeled tube. Tubes were then centrifuged at 2000 X g for 30 minutes at room temperature. Following centrifugation, the supernatant was decanted and 2 mls of Sample Diluent was pipetted into each tube which was then tightly recapped and vortexed for 5 seconds.

3. Specimens were placed in the manufacturer's Lysing rack as specified by a layout template. The Lysing rack was then placed into the BDProbeTec ET Lysing Heater for 30 minutes. Following heating, the rack was allowed to cool at room temperature for at least 15 minutes.

Following cooling, 150 µl of prepared specimen was dispensed into the manufacturer's Priming microwells, covered and incubated at room temperature for 20 minutes. The cover was then removed and the priming

microwells were placed in the BDProbeTec ET Priming and Warming Heater. The Amplification microwell plate, which corresponded to the priming plate, was immediately placed next to the priming microwells on the Priming and Warming Heater to pre-warm for 10 minutes. At the end of the 10 minute incubation, 100 µl of specimen was transferred from the priming microwells to the amplification microwells and sealed. The plate was then placed on the stage of the BDProbeTec ET Instrument where incubation and amplification occurred over a period of approximately 60 minutes. After completion of amplification, results were automatically printed from the instrument.

DEFINITIONS AND ANALYSES

1. CT Culture Positive

- A CT culture is considered positive if at least one inclusion is found after staining.

2. GC Culture Positive

- A GC culture is considered positive if the culture is presumably positive for gram-negative diplococci by gram stain, the colonies are oxidase positive, and the ID is confirmed by biochemical testing (Quad-Ferm) or species specific fluorescent staining.

3. Gold Standard

- For CT, the gold standard is defined as the combination of culture result and DFA result. For GC, the gold standard is culture.

4. “Enhanced” Gold Standard

- The “enhanced” gold standard is defined as diagnosis of infection using the combined criteria of culture, another amplified probe test (LCx), and DFA:
- For CT, a female patient is considered to be infected if the culture or DFA is positive, if both swab and urine are LCx positive, or if either specimen type is LCx positive and the DFA is positive.
- For GC, a female patient is considered to be infected if the culture is positive, or if both the swab and urine are LCx positive.

COMMENT. The BDProbeTec ET System for *N. gonorrhoeae* and *C. trachomatis* using a single swab specimen appeared to provide results which usually agreed with culture, or on occasion where cultures were negative and the BDProbeTec ET System were positive, could be verified as positive by Abbott LCx®.

BDProbeTec ET Assays for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* using urine specimens were found to contain inhibitory substances approximately 6% of the time.

CHLAMYDIA TRACHOMATIS

Female swab			
BD ProbeTec ET System vs. Culture/DFA			
		Culture/DFA	
		+	-
BDPT	+	54	13
Swab	-	4	392*
BDPT Swab Sensitivity = 54/56 (96.4%)			
BDPT Swab Specificity = 392/405 (96.8%)			
Swab Indeterminate Rate = 1/468 (0.2%)			
Swab Equivocal Rate = 6/468 (1.3%)			

*Two of these negative CT cultures were originally reported as positive due to a technical laboratory error in performance of the CT culture. These 2 cultures were repeated twice and were found to be culture negative as well as DFA ⊖, LCx ⊖.

Female urine			
BD ProbeTec ET System vs. Culture/DFA			
		Culture/DFA	
		+	-
BDPT	+	41	9
Urine	-	12	373*
BDPT Urine Sensitivity = 41/52 (80.4%)			
BDPT Urine Specificity = 373/382 (97.6%)			
Urine Indeterminate Rate = 26/460 (5.7%)			
Urine Equivocal Rate = 0/460 (0%)			

*Two of these negative CT cultures were originally reported as positive due to a technical laboratory error in performance of the CT culture. These 2 cultures were repeated twice and were found to be culture negative as well as DFA ⊖, LCx ⊖.

Female swab			
BD ProbeTec ET System vs. Culture/DFA/LCR			
		Culture/DFA/LCR	
		+	-
BDPT	+	55	12
Swab	-	6	388*
BecD Swab Sensitivity = 55/61 (90.2%)			
BecD Swab Specificity = 388/400 (97.0%)			
Swab Indeterminate Rate = 1/468 (0.2%)			
Swab Equivocal Rate = 6/468 (1.3%)			

Female urine			
BD ProbeTec ET System vs. Culture/DFA/LCR			
		Culture/DFA/LCR	
		+	-
BDPT	+	42	8
Urine	-	16	368*
BDPT Urine Sensitivity = 42/58 (72.4%)			
BDPT Urine Specificity = 368/376 (97.9%)			
Urine Indeterminate Rate = 26/460 (5.7%)			
Urine Equivocal Rate = 0/460 (0%)			

CHLAMYDIA TRACHOMATIS

BD ProbeTec ET System					
Summarized Sensitivity and Specificity					
		Sensitivity		Specificity	
Type	N=	Gold Culture/DFA	Enhanced Culture/DFA/LCR	Gold Culture/DFA	Enhanced Culture/DFA/LCR
Swab	468	96.4%	90.2%	96.8%	97.0%
Urine	460	80.4%	72.4%	97.6%	97.9%

NEISSERIA GONORRHOEAE

Female swab			
BD ProbeTec ET System vs. Culture			
		Culture	
		+	-
BDPT	+	52	8
Swab	-	2	403
BDPT Swab Sensitivity = 52/54 (96.3%)			
BDPT Swab Specificity = 403/411 (98.1%)			
Swab Indeterminate Rate = 1/471 (0.2%)			
Swab Equivocal Rate = 5/471 (1.1%)			

Female urine			
BD ProbeTec ET System vs. Culture			
		Culture	
		+	-
BDPT	+	47	4
Urine	-	6	376
BDPT Urine Sensitivity = 47/53 (88.7%)			
BDPT Urine Specificity = 376/380 (98.9%)			
Urine Indeterminate Rate = 29/463 (6.3%)			
Urine Equivocal Rate = 1/463 (0.2%)			

Female swab			
BD ProbeTec ET System vs. Culture/LCR			
		Culture//LCR	
		+	-
BDPT	+	56	4
Swab	-	2	403
BDPT Swab Sensitivity = 56/58 (96.6%)			
BDPT Swab Specificity = 403/407 (99.0%)			
Swab Indeterminate Rate = 1/471 (0.2%)			
Swab Equivocal Rate = 5/471 (1.1%)			

Female urine			
BD ProbeTec ET System vs. Culture/LCR			
		Culture//LCR	
		+	-
BDPT	+	50	1
Urine	-	7	375
BDPT Urine Sensitivity = 50/57 (87.7%)			
BDPT Urine Specificity = 375/376 (99.7%)			
Urine Indeterminate Rate = 29/463 (6.3%)			
Urine Equivocal Rate = 1/463 (0.2%)			

Summarized Sensitivity and Specificity					
BD ProbeTec ET System					
		Sensitivity		Specificity	
Type	N=	Gold Culture	Enhanced Culture/LCR	Gold Culture	Enhanced Culture/LCR
Swab	471	96.3%	96.6%	98.1%	99.0%
Urine	463	88.7%	87.7%	98.9%	99.7%

CONCLUSION

- The BDProbeTec ET System for detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in females using swabs is a promising alternative method to culture.
- In this study the presence of inhibitory substances in urine hindered testing of approximately 6% of specimens. Of the specimens which were not inhibited, there was, in general, good agreement between culture and the BDProbeTec ET System

