

Evaluation of BDProbeTec™ ET for the Detection of *Chlamydia Trachomatis* and *Neisseria Gonorrhoeae* in Female Urogenital Specimens

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INTRODUCTION

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (GC) are two of the most common sexually transmitted diseases (STD). Approximately four million new cases of CT and one million new cases of GC occur annually in the U.S. Asymptomatic infections are common in both men and women. Failure to provide treatment can lead to epididymitis in men and pelvic inflammatory disease and infertility in women. In addition, newborn infants exposed to CT during passage through an infected birth canal can develop chlamydial conjunctivitis and/or pneumonia. Currently, the Centers for Disease Prevention and Control recommends that sexually active teens and adults ≤ 24 years of age be routinely screened for both organisms. Thus, accurate laboratory tests for the diagnosis of chlamydia and gonorrhea are needed.

The "gold standards" for identification of CT or GC have been culture isolation. For CT, this procedure is costly and labor intensive. For gonorrhea, presumptive identification can be made by direct microscopic examination of Gram stained smears from symptomatic men. However, this method is not routinely used on women. The definitive diagnosis of *N. gonorrhoeae* is still the isolation of oxidase positive, Gram negative diplococci with confirmation by either DFA or sugar utilization tests. Introduction of direct fluorescent antibody (DFA), enzyme immunoassays (EIA) and direct DNA hybridization tests for these organisms have increased specimen turn around time, but these tests lack the performance profile of culture.

Recently, amplified DNA methods LCR (ligase chain reaction), PCR (polymerase chain reaction) and TMA (transcription mediated amplification) were FDA approved. Evaluations have shown these tests are highly sensitive and specific. However, each has some limitations. Among these are variable sensitivity to inhibitors, limited throughput that could restrict use in high volume laboratories, and labor intensiveness. Clearly, there is still a need for a sensitive and specific system to test large numbers of urogenital specimens for CT and GC.

We evaluated the BDProbeTec™ ET (BDPT, Becton Dickinson, Sparks, MD), a thermophilic strand displacement amplification assay for the identification of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* from a single specimen. The BDPT test contains an amplification control to detect inhibition. A multicenter evaluation was performed using the BDPT assay and Abbott LCx with female urogenital specimens. Results were compared to standard culture procedures.

MATERIALS AND METHODS

PATIENT POPULATION: Symptomatic and asymptomatic females were screened at family practice, teen, OB/GYN and STD clinics. Patients on antibiotic therapy were excluded from the evaluation. The study sites were located in Stockton, San Francisco and Cleveland. A total of 657 endocervical (cx) swabs and 588 first catch urine (FCU) were collected.

SPECIMEN COLLECTION: Female urogenital specimens were collected in the following order: 1) approximately 15-20 ml of FCU; 5 ml of the FCU was aliquotted into a sterile tube for LCx and a BDPT urine processing pouch was then placed into the FCU cup, 2) cx swabs for GC were streaked onto Thayer-Martin plates, and placed into candle jars, 3) randomized cx swabs for tissue culture (TC), BDPT and LCx were inserted into the appropriate collection media. All specimens were held at 4° C (except the GC plate).

GC ISOLATION: Inoculated Thayer-Martin plates were incubated at 36° C for 48 hours. Presumptive GC colonies were confirmed by DFA (Syva) and Quad Ferm Test (API).

CT ISOLATION: Using a modification of the procedure of Ripa and Mardh, cx swabs were inoculated within 72 hrs of collection. Each site used its usual and "optimal" method for chlamydia isolation rather than attempt to standardize methods. All sites used cycloheximide-treated McCoy cells; a blind pass and a DFA stain to identify inclusions in one-dram shell vials.

ABBOTT LCX ASSAY: All cx and FCU specimens were processed according to manufacturer's specifications within 4 days of collection. Cx swabs were heated at 97° C for 15 min. FCU aliquots of 1ml were cytospun for 15 min. The urine pellet was resuspended in LCx buffer and heated at 97° C for 15 min. For DNA amplification, 100 μ l of sample was added to a microfuge tube containing a predisposed LCx reaction mix of four oligonucleotide probes and a thermostable enzyme (ligase). The tubes were inserted into a Perkin Elmer thermocycler programmed for 40 cycles. A microparticle enzyme immunoassay was used to detect amplicons.

BDPT ASSAY: All cx and FCU specimens were processed within 4 days of collection. Cx swabs were transferred to a diluent tube, mixed, expressed and discarded. FCU aliquots of 4 ml were centrifuged at 2000 x g for 30 min. The urine pellet was resuspended in BDPT diluent and vortexed. Specimens and controls were placed on a lysing heater (114° C) for 30 min. Lysed samples were added to a microwell plate that contained GC, CT and amplification control (AC) primers and a fluorescent-labeled detector probe. After 20 min at rm temp, the plate was heated at 72.5° C for 10 min. Primed samples were then transferred to pre-warmed (54° C) microwell amplification plate containing DNA polymerase and restriction enzymes. The sealed plate was inserted into the BDProbeTec™ ET instrument (a thermally controlled fluorescent reader which monitored each reaction for the generation of amplified products). Total detection time for 32 specimens (each with results for CT, GC and AC) was 60 min.

TRUE POSITIVES (TP)+: True positives were defined as CT or GC culture positive. Specimens that were culture negative but BDPT and LCx positive were considered TP.

RESULTS

Figure 1: BDProbeTec ET System



Table 1

PERFORMANCE OF BDProbeTec™ ET FOR THE DIAGNOSIS OF C. TRACHOMATIS USING CERVICAL SPECIMENS (n=657)					
		True Positives*		Sensitivity	100.0%
		Pos	Neg	Specificity	99.3%
BDPT	Pos	33	4	+PV	89.2%
	Neg	0	609	-PV	100.0%

*True positives defined as TC positive or BDPT and LCx positive.

**11 BDPT specimens had equivocal results.

Table 2

PERFORMANCE OF LCx FOR THE DIAGNOSIS OF C. TRACHOMATIS USING CERVICAL SPECIMENS (n=657)					
		True Positives		Sensitivity	100.0%
		Pos	Neg	Specificity	99.7%
LCx	Pos	33	2	+PV	94.3%
	Neg	0	622	-PV	100%
				Prevalence	5.0%
				TC Sensitivity	69.7%

Table 3

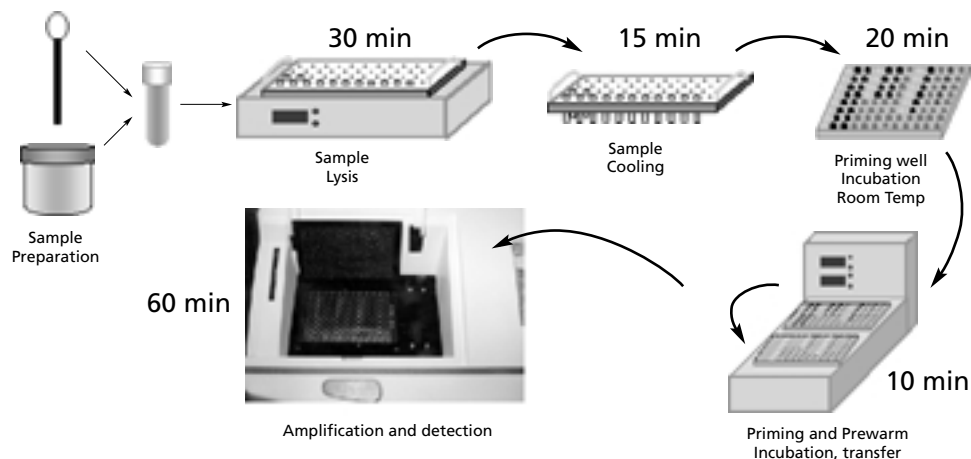
PERFORMANCE OF BDProbeTec™ ET FOR THE DIAGNOSIS OF C. TRACHOMATIS USING FCU SPECIMENS (n=588)					
		True Positives		Sensitivity	100.0%
		Pos	Neg	Specificity	98.6%
BDPT	Pos	32	7	+PV	82.0%
	Neg	0	513	-PV	100%

*36 BDPT specimens were inhibited.

Table 4

PERFORMANCE OF LCx FOR THE DIAGNOSIS OF C. TRACHOMATIS USING FCU SPECIMENS (n=588)					
		True Positives		Sensitivity	100.0%
		Pos	Neg	Specificity	99.5%
LCx	Pos	32	3	+PV	91.4%
	Neg	0	553	-PV	100%
				Prevalence	5.4%
				TC Sensitivity	68.7%

Figure 2: BDProbeTec ET Workflow



RESULTS

Table 5

PERFORMANCE OF BDProbeTec™ ET FOR THE DIAGNOSIS OF <i>N. GONORRHOEAE</i> IN CERVICAL SPECIMENS (n=644)					
		NG Culture		Sensitivity	100%
		Pos	Neg	Specificity	99.7%
BDPT	Pos	10	2	+PV	83.3%
	Neg	0	629	-PV	100%

*True positives defined as culture positive or BDPT and LCx positive.
 **3 BDPT specimens had equivocal results.

Table 8

PERFORMANCE OF LCx FOR THE DIAGNOSIS OF <i>N. GONORRHOEAE</i> IN FCU SPECIMENS (n=578)					
		NG Culture		Sensitivity	80.0%
		Pos	Neg	Specificity	99.6%
LCx	Pos	8	2	+PV	80%
	Neg	2	566	-PV	99.6%
				Prevalence	1.7%
				Culture Sensitivity	90%

Table 6

PERFORMANCE OF LCx FOR THE DIAGNOSIS OF <i>N. GONORRHOEAE</i> IN CERVICAL SPECIMENS (n=644)					
		NG Culture		Sensitivity	80.0%
		Pos	Neg	Specificity	100%
LCx	Pos	8	0	+PV	100%
	Neg	2	634	-PV	99.7%
				Prevalence	1.5%
				Culture Sensitivity	100%

Table 7

PERFORMANCE OF BDProbeTec™ ET FOR THE DIAGNOSIS OF <i>N. GONORRHOEAE</i> IN FCU SPECIMENS (n=578)					
		NG Culture		Sensitivity	90%
		Pos	Neg	Specificity	99.6%
BDPT	Pos	9	2	+PV	81.8%
	Neg	1	528	-PV	99.8%

*38 BDPT specimens were inhibited.

CONCLUSIONS

- Sensitivity and specificity of BDPT were outstanding for detection of CT and GC in female urogenital specimens.
- For CT detection, the BDPT was ~30% more sensitive than tissue culture isolation. For GC detection, the BDPT was comparable to culture. However, the number of GC positives evaluated was small.
- Results for the BDPT and LCx amplification assays were comparable for both CT and GC detection.
- BDPT inhibitors were found mostly (6.6%) in FCU specimens.
- BDPT is a rapid, easy method that would work well for screening large numbers of specimens. With high throughput, rapid turn around and excellent performance, it could replace TC, antigen detection and direct DNA hybridization methods, and be competitive with the currently available nucleic acid amplification assays.

