

Evaluation of the BDProbeTec™ ET Thermophilic Strand Displacement Assay for the Simultaneous Amplification and Detection of *Chlamydia Trachomatis* and *Neisseria Gonorrhoeae*.

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OBJECTIVE: To determine the performance characteristics of a real-time, semi-automated DNA amplification assay for the simultaneous detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC). Study assay results were compared to results obtained by both culture and ligase chain reaction (LCR).

METHODS: Swab and urine samples were collected from men attending STD clinics at each of three study sites. Becton Dickinson's semi-automated thermophilic strand displacement amplification assay was used to simultaneously amplify and detect CT and GC in the BD ProbeTec™ ET Systems (BD). An amplification control was run to rule out the presence of inhibitors within each specimen that might have prevented amplification. Urines were assayed by BD and LCR. Male urethral swabs were collected for BD and culture only; no LCR swab specimen was collected from males. Chlamydia and gonorrhea cultures were performed as previously described. LCR was performed according to the manufacturer's instructions.

RESULTS: Indiana University School of Medicine (IU), Johns Hopkins University (JHU), and University of Alabama-Birmingham (UAB) enrolled 294, 202 and 169 men respectively with a total of 665. Of the 665 patients, five could not be evaluated in the CT arm of the study due to missing comparator results. Men were defined as infected with gonorrhea if they had a positive culture. Men were defined as infected with chlamydia if they had a positive culture or 2 positive non-culture results. Since no LCR swab was collected, a positive swab or urine LCR and swab DFA were required in the absence of a positive culture. The CT and GC sensitivities were 94.6% and 98.4% respectively for both swab and urine specimens. The CT specificities were 94.5% and 91.4% for swab and urine specimens respectively. The GC specificities were 96.4% and 96.1% for swab and urine specimens respectively.

The CT assay had 29 swab positives and 45 urine positives not confirmed by cytospin DFA. The GC assay detected 17 positives from culture negative patients when swabs were used and 18 when urine samples were tested. The specificity determined for this assay was affected by not having an LCR swab result for comparison. If a positive patient was defined by a positive culture or a single positive LCR result, 16/29 swab CT results and 29/45 urine CT results classified as false positives would become confirmed positives. For GC 17/17 swab results and 14/18 urine results would change from false positives to confirmed positives. The resulting sensitivities and specificities respectively for CT were 95.2% & 97.5% for swabs and 95.6% & 96.8% urine. For GC, the adjusted sensitivities and specificities respectively were 98.6% & 100% for swabs and 98.5% & 99.1% for urine.

CONCLUSIONS: The BD system provides a rapid and sensitive test for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. The enclosed amplification and detection format, as well as the automation, make this assay potentially useful for the clinical laboratory setting.

INTRODUCTION

Chlamydia trachomatis (CT) is the most prevalent bacterial sexually transmitted disease (STD). The World Health Organization (WHO) estimates an annual worldwide incidence of 89 million cases of CT and 62 million cases of *Neisseria gonorrhoeae* (GC). Co-infections are common, occurring in 27-58% of patients tested for both organisms^(1,2). Both the high prevalence of CT and GC infections and the frequency of co-infections have increased the demand for more rapid and accurate diagnostic tests which can detect both organisms. Becton Dickinson's (BD) BDProbeTec™ ET System uses strand displacement amplification (SDA) to simultaneously amplify and detect CT and GC DNA. The BDProbeTec™ ET System was evaluated by comparing its performance with that of Ligase Chain Reaction (LCR) and culture on specimens collected from men attending an STD clinic.

SPECIMEN COLLECTION & REFERENCE TESTING

- One urethral swab was collected for inoculating the GC culture plate and was also used in the BDProbeTec™ ET GC Assay.
- A second urethra swab was collected for CT culture. A urine specimen was collected for both the LCR and BDProbeTec™ ET CT and GC assays.
- CT and GC cultures were performed as previously described.^(3,4)
- LCR was performed according to the package insert.

BDPROBETEC™ ET ASSAY

- BDProbeTec™ ET samples in lysis buffer were incubated at 110°C for 30 minutes in the manufacturer's Lysing rack.
- After incubation at room temperature for 15 minutes, samples were transferred to Priming microwells specific for CT, GC, or amplification control (AC) and incubated at room temperature for 20 minutes. Then both priming and amplification microwells were incubated at 72°C for 10 minutes.
- IMMEDIATELY after the 10-minute incubation samples were transferred from the Priming microwells to the preheated Amplification microwells. The sealed amplification microwells were placed into the BDProbeTec™ ET Instrument.
- A thermally controlled fluorescent reader incubates the reaction and monitors the real-time formation of amplification products. Results were calculated by a software algorithm and reported as positive, negative or indeterminate.

DATA

Performance of Swabs for Detection of CT					
	Culture or LCR & DFA		Culture or LCR		
	+	-	+	-	
BDPT	+	123	29	139	13
	-	7	501	7	501
n = 660	Sensitivity 94.6%		Specificity 95.2%		
	Sensitivity 94.5%		Specificity 97.5%		

Performance of Swabs for Detection of GC					
	Culture		Culture or LCR		
	+	-	+	-	
BD	+	188	17	205	0
	-	3	457	3	457
n = 665	Sensitivity 98.4%		Specificity 98.6%		
	Sensitivity 96.4%		Specificity 100%		

Performance of Urines for Detection of CT					
	Culture or LCR & DFA		Culture or LCR		
	+	-	+	-	
BD	+	122	45	151	16
	-	7	484	7	484
n = 658	Sensitivity 94.6%		Specificity 95.6%		
	Sensitivity 91.4%		Specificity 96.8%		

Performance of Urines for Detection of GC					
	Culture		Culture or LCR		
	+	-	+	-	
BD	+	187	18	201	4
	-	3	455	3	455
n = 663	Sensitivity 98.4%		Specificity 98.5%		
	Sensitivity 96.1%		Specificity 99.1%		

COMPARISON ANALYSIS

- Specimens obtained from culture negative patients which were positive by The BD ProbeTec™ ET assays were further analyzed by performing a cytospin on culture transport media and performing DFA on the sediment.
- Patients with GC infections were identified based on positive culture or positive LCx results.
- CT data were analyzed against two reference standards. Reference standard A required patients to have a positive culture or both positive LCx and DFA results to qualify as infected.
- Due to the low sensitivity of DFA compared to LCR, reference standard B defined positives based on either a positive culture or a positive LCx result, eliminating the DFA requirement.
- 665 males were enrolled.
 - For the analysis of CT results, data were unavailable for 5 swab specimens and 7 urine specimens.
 - For analysis of the GC results, data were unavailable for 5 swab specimens and 2 urine specimens.
- Analysis of CT data against reference standard A identified 130 positive patients (19.7%).
 - Urine samples tested by The BDProbeTec ET CT assay identified 122/129. 45 urine positives were not confirmed by cytospin DFA. (unconfirmed positives = 8.6%)
 - Swab samples tested by The BDProbeTec ET CT assay identified 123/130. 29 swab positives were not confirmed by cytospin DFA. (unconfirmed positive = 5.5%)
 - 12 urine samples and no swab specimen were classified as Indeterminate due to AC failure.
- Analysis of CT data against reference standard B identified 158 positive patients (23.9%).
 - Urine samples tested by The BDProbeTec ET CT assay identified 151/158. 16 BD urine positives were not culture or LCx positive (unconfirmed positive = 3.2%).
 - Swab samples tested by The BDProbeTec ET CT assay identified 139/146. 13 BD swab positives were not culture or LCx positive (unconfirmed positive = 2.5%).
- Analysis of GC data on the basis of culture results alone resulted in 191 positive patients (28.7%).
 - Urine samples tested by The BDProbeTec ET GC assay identified 187/190. 18 urine positives were not culture positive (unconfirmed positive = 3.9%).
 - Swab samples tested by The BDProbeTec ET GC assay detected 188/191. 17 swab positives were not culture positive (unconfirmed positive = 3.6%).
 - 17 urine samples and no swab specimen were classified as Indeterminate due to AC failure.
- When GC positives were defined by either culture or LCx positivity, 208/665 positive patients were identified. (31.3%).
 - Urine samples tested by The BDProbeTec ET GC assay identified 201/204. 4 BD urine positives were not confirmed by culture or LCx (unconfirmed positive = 0.9%)
 - Swab samples tested by The BDProbeTec ET GC assay detected 205/208. There were no unconfirmed swab positives.

CONCLUSION

- Two reference standards were used in the analysis of CT. The sensitivity of DFA, and the comparability of urethral swab to urine specimens, create artificially low specificity in any assay being evaluated. While the cytospin DFA resolution of discordant results is often used as a standard, acceptance of the high sensitivity and specificity of the FDA cleared nucleic acid amplification tests has made LCx a valid reference standard in the absence of DFA.
- The stability of both reagents and samples at room temperature facilitates collection of specimens in non-clinic settings and transport to the laboratory.
- An amplification control is available with this assay to allow the occurrence of inhibitors to be monitored. In this study population, inhibitory samples composed only 1.8% for CT and 2.6% for GC of the total tested.
- The BDProbeTec™ ET System demonstrated high-throughput screening with high sensitivity and specificity for both CT and GC. The assay is more sensitive than culture in identifying CT and GC.

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