

Evaluation of Room Temperature Storage and Stability of Specimens in the BDProbeTec™ ET System for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* from Female Endocervical and Female and Male Urine Specimens

RUTH KENDRICK¹, C CAMMARATA², DH MARTIN², RL SAUTTER³, T RUDOLPH⁴, WD LeBAR¹

HCL-Providence Hospital, Southfield, MI¹, LSU Medical Center, New Orleans, LA², Pinnacle Health Systems, Harrisburg, PA³, Detroit Health Department, Detroit, MI⁴

Abstract

■ The Becton Dickinson BDProbeTec™ ET System is a new instrumented system which uses isothermal strand displacement DNA amplification for the detection of CT and GC. The system is based on simultaneous amplification and detection of target DNA by the use of amplification primers along with a fluorescent labeled detector probe. This study determined the room temperature storage and transport of female endocervical, female and male urine specimens. Initial BDPT results were also compared to LCR for CT and GC assays (Abbott Diagnostics). Specimens were obtained from patients attending an inner city STD clinic. Five endocervical swabs (4 BDPT, 1 LCR) and 1 urine specimen were obtained from 100 women. Urine specimens were also obtained from 40 male patients. BDPT and LCR assays were processed within 24 hours of collection. The remaining BDPT specimens were held at room temperature for 2, 4 and 5 days. Results of testing at additional time points were compared to the original BDPT results. Overall, there was 94% agreement of BDPT and LCR for the detection of CT and 98% agreement for the detection of GC. Holding specimens at room temperature from 2 to 5 days resulted in disagreement with initial BDPT swab results in 0 to 7% of cases for CT and 0 to 4% for GC. Disagreement with urine results occurred in 0 - 4% for CT and 0 - 3% for GC. The BDPT correlates well with LCR for the detection of CT and GC from endocervical and urine specimens. Swab and urine samples tested on the BDProbeTec™ ET are stable at room temperature for up to 5 days. Additional studies are ongoing.

INTRODUCTION

The BDProbeTec™ ET (BDPT) System is a new DNA amplification methodology which uses homogeneous strand displacement amplification (SDA) with an energy transfer detection chemistry for the detection of *C. trachomatis* (CT) and *N. gonorrhoeae* (GC) in urogenital specimens.

The purpose of this study was to compare the BDPT to LCR and to verify room temperature (18 - 30°C) storage and transport for female endocervical swabs and for male and female urine specimens after addition of the Urine Preservative Pouch (UPP) for up to 5 days after collection.

MATERIALS AND METHODS

PATIENTS: Specimens were obtained from patients attending a STD clinic with clinical or epidemiological history suggestive of chlamydial or gonococcal infection. A swab for the LCR assay and 4 swabs for the BDPT assay were collected from the endocervix of 100 women to date. A voided urine sample was also collected on all female patients. Urine specimens were obtained from 39 men. Specimens were randomized and processed as below.

ENDOCERVICAL SPECIMENS: If the patient met the inclusion criteria, one swab for LCR and 4 swabs for BDPT were collected. BDPT swabs were randomized and labeled "A", "B", "C", and "D". Swabs labeled "A" were stored at room temperature for <24 hours and used for comparison to LCR. Swabs B-D were used in the stability study and stored as shown in Table 1.

URINE: Patients were instructed to collect 30 - 90 ml of voided urine. After mixing 5 ml urine was transferred into a conical tube and the UPP was added to the remainder of the urine. Samples for LCR were transported at 2 - 8° C and BDPT at room temperature. A sample was tested by BDPT and LCR within 24 hours of receipt. The remainder of the urine was stored at room temperature and used in the stability study as shown in Table 1.

STABILITY STUDY: Ten urine and 10 swab specimens positive for CT and / GC were held at room temperature and tested at the intervals below. Swab specimens negative for CT (N=21) and GC (N=25) and 25 urine samples were also included in the comparison.

Table 1

Length of room temperature storage of urine and swab specimens prior to processing and testing by BDPT	
Swab/ Urine	Time
Sample A	<24 hours
Sample B	2 days
Sample C	4 days
Sample D	5 days

LCR: The LCR assays (Abbott Diagnostics) for detection of CT and GC were performed according to manufacturer's instructions.

BD-PT: Endocervical, urethral and urine samples were processed according to manufacturer's instructions. The BD-PT CT and GC amplified DNA assays are based on the simultaneous amplification and detection of target using amplification primers and a fluorescent labeled detector probe. The SDA reagents are dried in two separate microwell strips. The processed sample is added to the priming microwell which contains the amplification primers, labeled detector probe, and other reagents necessary for amplifi-

cation. After two incubation steps, the reaction mixture is transferred to the amplification microwell which contains a DNA polymerase and restriction enzyme necessary for SDA. The microwells are then incubated in a thermally controlled reader which monitors each reaction for the generation of amplified products. An amplification control is run with each specimen. Results are reported through an algorithm as positive, negative or indeterminate.

Results of the comparison with LCR were analyzed as percent agreement while results in the stability study were analyzed as percent agreement compared to Day 0 results (Sample A).

RESULTS

Comparison of LCR and BDPT									
	CT (Swab)					CT (Urine)			
	+	-	+	-		+	-	+	-
BD-PT	+	-	+	-	BD-PT	+	-	+	-
LCR	+	+	-	-	LCR	+	+	-	-
	10	4	3	78		13	1	5	98

	GC (Swab)			GC (Urine)	
	+	-		+	-
BD-PT	+	-	BD-PT	+	-
LCR	+	-	LCR	+	-
	11	83		10	99

Agreement	CT		GC	
	Swabs	Urines	Swabs	Urines
BD-PT vs LCR	92.6%	94.8%	100%	95.1%
Overall Agreement	CT= 94%		GC = 98%	

Stability				
% Correct Results Compared to Day 0				
	CT (Swab)	CT (Urine)	GC(Swab)	GC(Urine)
Day 2	90.3%	86.5%	91.4%	88.6%
Day 4	90.3%	94.6%	97.1%	91.4%
Day 5	96.8%	89.2%	94.3%	80%

% Incorrect Results Compared to Day 0				
	CT (Swab)	CT (Urine)	GC(Swab)	GC(Urine)
Day 2	3.2%	2.7%	2.9%	0%
Day 4	6.5%	0%	0%	0%
Day 5	3.2%	0%	0%	2.9%

% Indeterminate Compared to Day 0				
	CT (Swab)	CT (Urine)	GC(Swab)	GC(Urine)
Day 2	0%	5.4%	2.8%	11.4%
Day 4	0%	5.4%	2.8%	8.6%
Day 5	0%	10.8%	0%	17.1%

CONCLUSIONS

The results of this study demonstrate that the BDProbeTec™ ET correlates well with LCR for the detection of CT and GC from endocervical and urine samples. Swab specimens are stable at room temperature for up to 5 days. Urine reportable results are slightly decreased over time.