

# Evaluation of the BD ProbeTec ET System for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections of the Endocervix and Oropharynx in Women

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## ABSTRACT

**OBJECTIVES:** To determine the performance of a real-time DNA amplification assay, BD ProbeTec ET System (BDPT, BD Diagnostic Systems), for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) from both endocervical and oropharyngeal samples.

**METHODS:** Swab samples were collected from women attending an OB/GYN clinic at each of six study sites. Endocervical swab samples were assayed by BDPT and Amplicor (AMP, Roche). Oropharyngeal swab samples were assayed by BDPT and DNA probe (Gen-Probe). For the oropharyngeal swab samples, discordant results for CT were confirmed by AMP and discordant results for GC were confirmed by established PCR methods as follows, cpp B PCR, nested cpp B PCR, and 16S rRNA PCR.

**RESULTS:** A total of 364 endocervical and 247 oropharyngeal specimens were collected under informed consent from 364 patients. The agreement rates of the BDPT and AMP assays for the detection of CT and GC from endocervical samples were 99.2% (361/364) for CT and 99.5% (362/364) for GC. For oropharyngeal swabs the BDPT yielded 21 CT positives. Of these, 19 samples were CT negative by DNA probe. Using AMP, 16/19 (84.2%) of the BDPT+/DNA probe-samples were positive. The BDPT also yielded 21 GC positives of which 15 samples were negative by DNA probe. After additional testing 14/15 BDPT+/DNA probe-samples (93.3%) were positive by at least two of the PCR methods.

**CONCLUSIONS:** The BDPT performs comparably to AMP for detection of CT and GC from endocervical swab samples. Because the DNA probe method is low in sensitivity, the BDPT yielded several additional positive results on oropharyngeal swab samples. Further analysis using alternative methods of DNA amplification illustrated the BDPT to be more sensitive than DNA probe and that it may be clinically useful for the detection of CT and GC from oropharyngeal samples.

## INTRODUCTION

*Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) are among the major causes of sexually transmitted diseases (STD) in the world. In Japan, CT urogenital infection is the most prevalent STD with an incidence rate that has steadily increased until peaking in 2002 and has remained relatively constant.

Consistent with increased sexual activity, the incidence rate in Japan is the highest for men and women in their twenties. Even among teens, the incidence rate for this age group comprises 20% of all CT infections and represents a population where intervention efforts are necessary. Among men, CT and GC are associated with urethritis and epididymitis which are often symptomatic and associated with pain on urination and purulent secretions. However, in women, most infected individuals are asymptomatic. If untreated, the infection may evolve into pelvic inflammatory disease and may result in serious sequelae, such as ectopic pregnancy and infertility as well as perihepatitis.

With increased public tolerance of different sexual practices as well as the growth of the commercial sex industry in Japan providing oral sex services, the incidence of oral-pharyngeal CT and GC infections have increased to the extent that oral-pharyngeal infections are seen as a major factor in promoting the spread of infections which are often asymptomatic.

A number of highly sensitive nucleic acid amplification assays have been commercially developed for the detection of CT and GC from endocervical swabs and first catch urine. However, the Amplicor STD-1 (Roche), which is the nucleic acid amplification method most commonly used in Japan, has been reported to cross-react with commensal oral *Neisseria* resulting in false positive GC results. For this reason, no nucleic acid amplified method for detection of GC from the oropharynx is available for testing oropharyngeal specimens in Japan.

In this study, we evaluated the BD ProbeTec system (BDPT, BD Diagnostic Systems), the newly nucleic acid amplified system for detection of CT and GC, using both endocervical and oropharyngeal samples.

## MATERIALS AND METHODS

### Patients

- No. of patients: 307
  - CSW: 171
  - General female: 136
- Mean age: 26.9 ± 5.8 years

### Specimens

- Endocervical specimen: 364 samples
- Oropharyngeal specimen: 247 samples

### Assays

- (1) BD ProbeTec ET CT/GC (BD Diagnostic Systems)
  - Target DNA
  - *C. trachomatis*: Chlamydial cryptic plasmid
  - *N. gonorrhoeae*: Chromosomal pilin gene-inverting protein homologue
  - Amplification: Simultaneous strand displacement amplification (SDA)
- (2) Amplicor STD-1 (Roche)
- (3) DNA probe: Pace 2 (Gen-Probe)

### Sample preparation and Detection Assay of BDPT

- Lysing of the target organism in the specimen: Heat at 114°C for 30 min
- Cool at room temperature for at least 15 min.
- Transfer 150 µL of processed sample to Priming Microwells
- Sit at room temperature at least 20 min.
- Place Priming Microwells and Amplification Microwells in Priming/Warming Heater
- Incubate for 10 min.
- Transfer 100 µL to Amplification Microwells
- Seal Plate and place Amplification Microwells into instrument
- Activate automated amplification and detection of target DNA
- Testing completion time: 60 min.
- Total time to results: 2 hours

### Confirmatory methods\*1 for *N. gonorrhoeae* detection

The specificity of the BDPT *N. gonorrhoeae* positive result was evaluated with the three established PCR assays, *cppB* PCR, Nested *cppB* PCR, 16S rRNA PCR.

## RESULTS

### Endocervical specimens

Table 1. Agreement of *C. trachomatis* results between BDPT and AMP on the endocervical specimens

<i>C. trachomatis</i>		BD ProbeTec ET	
		Positive	Negative
Amplicor PCR	Positive	55	1
	Negative	2	306

Agreement: 99.2% (361/364)

Table 2. Agreement of *N. gonorrhoeae* results between BDPT and AMP on the endocervical specimens

<i>N. gonorrhoeae</i>		BD ProbeTec ET	
		Positive	Negative
Amplicor PCR	Positive	10	0
	Negative	2 (*2)	352

Agreement: 99.5% (362/364)

### Oropharyngeal specimens

Table 3. Agreement of *C. trachomatis* results between BDPT and DNA probe on the oropharyngeal specimens

<i>C. trachomatis</i>		BD ProbeTec ET	
		Positive	Negative
DNA Probe	Positive	2	0
	Negative	19 (*3)	226

\*3: Sixteen out of 19 samples were positive by Amplicor PCR

Table 4. Agreement of *N. gonorrhoeae* results between BDPT and DNA probe on the oropharyngeal specimens

<i>N. gonorrhoeae</i>		BD ProbeTec ET	
		Positive	Negative
DNA Probe	Positive	6	0
	Negative	15 (*4)	226

### Confirmatory results (\*2, \*4)

	*2		*4														
	1	2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<i>cppB</i> PCR	P	N	P	P	P	P	P	P	N	N	N	N	N	N	N	N	N
Nested <i>cppB</i> PCR	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
16S rRNA PCR	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N

P: Positive, N: Negative

\*1: David J Farrell, Journal of Clinical Microbiology 37(2), p386-390, 1999  
David J Diemert et al., Journal of Clinical Microbiology 40(11), p4056-4059, 2002

### CONCLUSIONS

- The BDPT performs comparably to AMP for detection of CT and GC from endocervical swab samples.
  - For 15 discrepancy GC results on oropharyngeal specimens, 14 out of 15 are positive at least two alternative methods. These are considered a true positive.
  - In our preliminary unpublished study using adult male oropharyngeal specimens, we did not observe cross-reaction with *Neisseria* other than *N. gonorrhoeae*.
  - Because the DNA probe method is low in sensitivity, the BDPT yielded several additional positive results on oropharyngeal swab samples.
  - The BDPT may be clinically useful for the detection of CT and GC from oropharyngeal samples.
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