

Experimental Evaluation of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Detection Sensitivity of BDProbeTec™ ET

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ABSTRACT

■ *In vitro* detection sensitivity of BDProbeTec™ ET(PT), which is based on SDA, a new nucleic acid amplification procedure, was tested for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* and was compared with that of the AMPLICOR (AMP) method for *C. trachomatis* (CT) and *N. gonorrhoeae* (NG). *N. gonorrhoeae* ATCC 49226 was used for a test strain and the urine specimens presented with *C. trachomatis* or *N. gonorrhoeae* were used.

For the detection sensitivity comparison of both methods, *N. gonorrhoeae* was added to sterile urine to a final concentration of 10³ CFU/mL and a 10-fold dilution series of the urine was then prepared with sterile physiological saline. Similarly, a 10-fold dilution series of *C. trachomatis* and *N. gonorrhoeae* positive patient urine was prepared and used for a sensitivity comparison test.

Three samples of up to 10-fold dilution of *N. gonorrhoeae* added urine showed positive and the 100-fold dilution of the samples showed negative both in the PT and AMP NG methods. In 5 *C. trachomatis* positive patient urine specimens, PT showed positive in 1 sample of 1000-fold dilution, 2 samples of 100-fold dilution and 2 samples of 10-fold dilution, and AMP CT showed positive in 3 samples of 100-fold dilution and 2 samples of 10-fold dilution. In 5 *N. gonorrhoeae* positive patient urine specimens, both the PT and AMP NG methods showed positive in 3 samples of 1000-fold dilution and 2 samples of 100-fold dilution.

From these results, it can be confirmed that BDProbeTec™ ET based on the SDA method has equal or superior sensitivity in the detection of *C. trachomatis* and *N. gonorrhoeae* as compared with AMPLICOR based on the PCR method and is useful for rapid detection of these microorganisms.

INTRODUCTION

Chlamydia trachomatis and *Neisseria gonorrhoeae* infections are major sexually transmitted diseases in Japan. In recent years, the rate of chlamydia infection and gonococcal infection have reached 32.22% and 11.85%, respectively. It is said especially that gonococcal infections have been increasing since the mid-1990s.

The BDProbeTec™ ET System developed by Becton Dickinson utilizes SDA (Strand Displacement Amplification) technology for the simultaneous amplification and detection of *C. trachomatis* and *N. gonorrhoeae* DNAs. The system is easier to use than with conventional detection systems using genes, and provides improved sensitivity and specificity.

In this study, the BDProbeTec™ ET System (PT) was compared with the AMPLICOR STD-1 (AMP) *Chlamydia trachomatis* and *Neisseria gonorrhoeae* to test the detection sensitivities of *C. trachomatis* and *N. gonorrhoeae* from urine specimens and the ability to detect *N. gonorrhoeae* from potentially applicable pharynx specimens.

MATERIALS AND METHODS

SPECIMENS

■ For testing of detection sensitivity in urine specimens

N. gonorrhoeae ATCC 49226 strain was added to sterilized urine specimens to make a final concentration of 10³CFU/mL. The suspension was diluted with sterile physiological saline to prepare 10-fold serial dilutions. Then, each dilution was divided into two samples for testing with the BDProbeTec™ ET System and the AMPLICOR. Also, 5 *C. trachomatis* or *N. gonorrhoeae* positive urine specimens were diluted with sterile physiological saline to prepare 10-fold serial dilutions. Then, each dilution was divided into two samples for testing with the BDProbeTec™ ET System and the AMPLICOR.

■ For detection of *N. gonorrhoeae* from saliva specimens

Saliva specimens collected from 10 adult healthy volunteers were used for *N. gonorrhoeae* detection with the BDProbeTec™ ET System and the AMPLICOR. At the same time, quantitative cultures were made with these saliva specimens to detect normal oral bacterial flora. In addition, *N. gonorrhoeae* ATCC 49226 was added to saliva specimens, which were interpreted as *N. gonorrhoeae* negative with the AMPLICOR, to make a final concentration of 10² and 10⁴ CFU/mL. These suspensions were tested by both methods to detect *N. gonorrhoeae*.

METHODS

■ BDProbeTec™ ET System

The BDProbeTec™ ET System *C. trachomatis* and *N. gonorrhoeae* (Becton Dickinson) was used. The BDProbeTec™ ET Urine Processing Pouch was added to urine specimens and allowed to sit at room temperature for 2 hours or more. Then, 4 mL of the specimen was transferred into a centrifugation tube, which was centrifuged at 2000 x g for 30 minutes at room temperature. After centrifugation, 2 mL of Sample Dilution was added to the sediment and mixed on a vortex mixer.

Saliva specimens soaked in Culturette™ Direct (Becton Dickinson) were added to 2 mL of Swab Dilution and mixed.

These urine and saliva specimens were incubated on the Lysing Heater at 114°C for 30 minutes and were then allowed to cool at room temperature for 15 minutes. One hundred and fifty µL of the specimens were dispensed to each well of the Priming Microwells and allowed to sit for 20 minutes. The Priming Microwells were transferred to the Priming Heater (72.5°C) for 20-minute incubation. After incubation, 100 µL of the specimens were transferred to each well of the Amplification Microwells which had been preincubated (54°C) for 10 minutes. The Amplification Microwells were sealed and immediately placed into the BDProbeTec™ ET System for testing.

■ AMPLICOR STD-1

AMPLICOR STD-1 *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (Roche Diagnostic Systems) were processed according to manufacturer's instructions.

■ Quantitative culture of saliva and bacterial isolation for identification

A 10-fold serial dilution of the saliva was prepared. Each 50 µL aliquot of the saliva suspension was then spread on Chocolate II Agar (Becton Dickinson) plates using a glass stick. The Chocolate II Agar plates were incubated at 35°C for 48 hours in 10% CO₂. After incubation, the number of colonies was counted on plate. *Neisseria* counts in the saliva were confirmed using the VITEK system (bioMérieux).

RESULTS

■ Detection sensitivities of *C. trachomatis* and *N. gonorrhoeae* from urine specimens

The urine specimens to which *N. gonorrhoeae* ATCC 49226 strain was added to make a final concentration of 10³CFU/mL were positive in the 10-fold dilution but negative in the 100-fold dilution in both methods (Table 1). Of 5 *N. gonorrhoeae* positive patient urine specimens, 3 specimens up to 1000-fold dilution and 2 specimens up to 100-fold dilution showed positive in both methods (Fig. 1). In *C. trachomatis* positive patient urine specimens, 1 specimen in 1000-fold dilution, 2 specimens in 100-fold dilution, and 2 specimens in 10-fold dilution showed positive with the BDProbeTec™ ET System, whereas 3 specimens in 100-fold dilution and 2 specimens in 10-fold dilution showed positive with the AMPLICOR (Fig. 2).

■ Detection of *N. gonorrhoeae* from saliva specimens and influence of the oral bacterial flora

With the AMPLICOR, false positive caused by cross reaction with other *Neisseria* was observed in 5 of 10 specimens. With the BDProbeTec™ ET System, all specimens were negative with no influence from other *Neisseria*. Five negative saliva specimens containing 10⁴CFU/mL of *N. gonorrhoeae* with the AMPLICOR showed positive in both methods, and one specimen in 10² CFU/mL showed positive in both methods. In the saliva culture in this study, 10⁴–10⁶ CFU/mL of normal oral *Neisseria* flora were detected. (Table 2,3).

Table 1. Sensitivities of PT and AMP to *N. gonorrhoeae* ATCC 49226 in sterile urine

Sample no.	Reaction in dilution series					
	x1 ^a		x10		x100	
	PT ^b	AMP ^c	PT	AMP	PT	AMP
	+ 46050	+ >3.000	+ 2866	+ 2.067 1.815	- 172	- 0.055
	+ 56624	+ >3.000	+ 2942	+ 1.994 2.057	- 487	- 0.067
	+ 61728	+ >3.000	+ 14006	+ 1.910 2.120	- 378	- 0.081
Control ^d	+ 66281	+ >3.000	+ 13874	+ 1.382 2.143	- 545	- 0.149

^a Number of *N. Gonorrhoea* ATCC49226: 9.6x10³CFU/mL

^b MOTA

^c OD 450nm

^d Sterile physiological saline

Table 2. Reactivity of PT and AMP, and number of *Neisseria* in saliva

Saliva no.	PT	AMP	Total viable cells (CFU/mL)	Culture result (CFU/mL)
1	—	—	4.2x10 ⁴	<i>N. sicca</i> 2.0x10 ⁵ <i>N. subflava</i> 8.0x10 ⁴
2	—	+	2.2x10 ⁴	<i>Neisseria</i> sp. 2.0x10 ⁴
3	—	+	8.0x10 ⁷	<i>Neisseria</i> sp. 6.6x10 ⁵
4	—	—	6.0x10 ⁷	<i>Neisseria</i> sp. 5.0x10 ⁶
5	—	—	3.2x10 ⁴	<i>N. subflava</i> 4.0x10 ⁶ <i>Neisseria</i> sp. 6.0x10 ⁴
6	—	+	1.0x10 ⁷	<i>Neisseria</i> sp. 2.0x10 ⁶
7	—	+	4.6x10 ⁴	<i>N. sicca</i> 1.2x10 ⁶
8	—	—	4.0x10 ⁷	<i>N. sicca</i> 2.8x10 ⁶
9	—	+	8.0x10 ⁷	<i>Neisseria</i> sp. 4.0x10 ⁵
10	—	—	2.0x10 ⁷	<i>N. sicca</i> 1.0x10 ⁶ <i>Neisseria</i> sp. 4.0x10 ⁵

Table 3. Recoveries of *N. gonorrhoeae* in saliva by PT and AMP

Saliva + <i>N. gonorrhoeae</i>						
Saliva no.	Saliva		10 ² CFU/mL		10 ⁴ CFU/mL	
	PT	AMP	PT	AMP	PT	AMP
1	—	—	—	—	+	+
4	—	—	+	—	+	+
5	—	—	—	—	+	+
8	—	—	—	—	+	+
10	—	—	—	+	+	+

Figure 1. Results of PT and AMP of serial dilutions of patients urine with *N. gonorrhoeae* infections

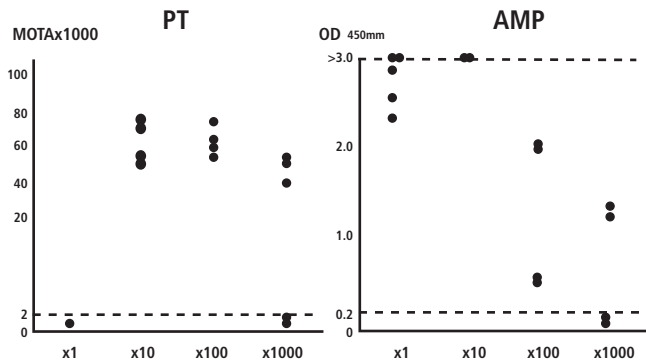
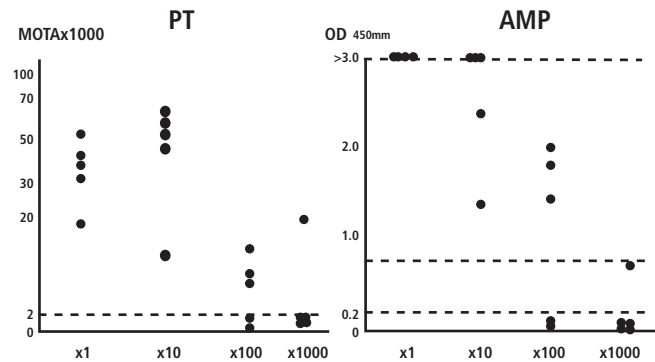


Figure 2. Results of PT and AMP of serial dilutions of patients urine with *C. trachomatis* infections



DISCUSSION AND CONCLUSION

Detection sensitivities of *N. gonorrhoeae* from *N. gonorrhoeae*-added urine specimens were almost equivalent in interpretive results from both the BDProbeTec™ ET System and the AMPLICOR. The detection sensitivities were 96 CFU/mL (19.2 CFU/assay) with the BDProbeTec™ ET System and 96 CFU/mL (4.8 CFU/assay) with the AMPLICOR. Both methods have the equivalent ability to detect *C. trachomatis* from *C. trachomatis* positive patient urine specimens.

In the *N. gonorrhoeae* detection of saliva specimens, *N. gonorrhoeae* was detected with the BDProbeTec™ ET System without cross-reaction with oral *Neisseria* flora which was observed with the AMPLICOR.

It was confirmed that the BDProbeTec™ ET System, a novel amplified DNA method based on SDA, was equivalent to the PCR method in the ability to detect *N. gonorrhoeae* and *C. trachomatis* from STD patient specimens.

The BDProbeTec™ ET System does not require the thermalcycler and enables the performance of rapid reactions under a single temperature condition. In addition, the system minimizes contamination by its closed system and is useful for clinical laboratories because no extra testing area is necessary.

