

# Comparison of the BD ProbeTec ET and Gen Probe PACE 2 Assays for the Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Endocervical Samples

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## REVISED ABSTRACT

*Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are the most common bacterial sexually transmitted diseases in the United States and worldwide. In women, untreated infections have been linked to infertility, pelvic inflammatory disease, and ectopic pregnancy. Recovery of these organisms in culture depends on many variables. In response to the shortcomings of culture, non-culture assays have been developed that detect organism nucleic acid. First generation assays, such as the Gen Probe PACE 2 (PACE 2), detect organism-specific DNA sequences. Recently developed assays utilize nucleic acid amplification to amplify and detect target DNA. One such assay, BD ProbeTec ET (ProbeTec), utilizes strand displacement amplification coupled with fluorescent energy transfer to detect amplified DNA. A study was undertaken to evaluate the performance of ProbeTec compared with the currently used PACE 2 assay for detection of CT and NG in endocervical specimens. A total of 467 endocervical swabs were collected in duplicate and tested by both PACE 2 and ProbeTec. A total of 46 (10.0%) samples were positive for CT and 9 (1.9%) samples were positive for NG. Of the 46 specimens positive for CT, 32/46 (69.6%) were positive by both assays and 14/46 (30.4%) were positive by ProbeTec alone. Of the 9 specimens positive for NG, 8/9 (88.9%) were positive by both assays and 1/9 (11.1%) was positive by ProbeTec alone. None of the samples were positive for CT or NG by PACE 2 alone. In order to determine the reproducibility of positive ProbeTec results, 49 CT positive specimens (24 low positive and 25 high positive) and 32 NG positive specimens (7 low positive and 25 high positive) were retested. A total of 13/24 (54.2%) low positive CT specimens and 4/7 (57.1%) low positive NG specimens repeated positive. All CT and NG high positive specimens remained positive after repeat testing. These results indicate that ProbeTec detected 30.4% and 11.1% more CT and NG positive specimens, respectively. Therefore ProbeTec is more sensitive than PACE 2 for the detection of CT and NG in endocervical specimens. In addition, only low positive ProbeTec results require repeat testing.

## INTRODUCTION

*Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) infections are the most common bacterial sexually transmitted infections in the United States and worldwide. Both organisms produce insidious infections in women that may lead to serious complications such as pelvic inflammatory disease, infertility and ectopic pregnancy. In addition, infants born vaginally to infected mothers are at increased risk of acquiring infection. Untreated CT infection may also increase the risk of invasive cervical cancer.

The current reference standard for the detection of these organisms is culture. However, CT can only be cultivated in tissue culture and NG is a bacterium that requires specialized growth media and conditions. In addition, while the major advantage of culture is specificity, culture sensitivity is only 70–90%. Thus the identification of infected individuals is hampered by the unavailability of sensitive culture systems. In response to the shortcomings of culture, non-culture assays have been developed that detect nucleic acid and do not require viable organisms. Non-amplified nucleic acid based assays such as the Gen Probe PACE 2 assay (PACE 2) use nucleic acid hybridization techniques to detect rRNA. In an effort to further improve the detection of these pathogens, nucleic acid amplification assays have been developed. These amplification assays are reported to be more sensitive than non-amplification DNA probe assays. One such assay, the Becton Dickinson ProbeTec ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assay (ProbeTec), uses strand displacement amplification coupled with fluorescent energy transfer to detect CT and NG. The increased sensitivity of these assays has the potential to significantly influence patient outcomes.

A study was undertaken to evaluate the performance of ProbeTec compared with the currently used PACE 2 assay for detection of CT and NG in endocervical specimens.

## MATERIALS AND METHODS

**PATIENT POPULATION.** Endocervical swabs were collected from 467 women from July through September 2002 presenting to the OB/GYN clinic at Shands Jacksonville for routine gynecologic or obstetric care and who required testing for *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*. The study was performed with the approval of the institutional review board. Verbal consent was obtained from each patient.

**SPECIMEN COLLECTION.** Samples were collected after the cervical os was cleaned to remove excess mucus and exudates. Two specimens were collected from each patient as follows: (i) for the PACE 2 assay, the swab provided in the PACE 2 collection kit was placed in the PACE 2 transport tube and submitted to the laboratory at room temperature. Upon receipt in the laboratory, the tube was vortexed, the swab was removed and the specimen was stored at room temperature until processing; (ii) for the ProbeTec assay, the swab provided in the ProbeTec collection kit was placed in the ProbeTec transport sleeve and submitted to the laboratory at room temperature. The specimen was stored at room temperature until processing. Immediately prior to testing, the swab was placed in ProbeTec specimen diluent, was vigorously mixed and removed. To prevent sampling bias, the order of specimen collection was randomized. All samples were tested within 48 hours of receipt in the laboratory.

**PACE 2 ASSAY.** Specimens were processed and assayed according to the manufacturer's instructions (Gen Probe, San Diego, CA). A PACE 2 result was considered positive if the difference between the patient's result and the mean of the negative references was  $\geq 300$  relative light units (RLU). Patient specimens with results between 300 and 999 RLU were repeated prior to reporting. Following testing, processed specimens were stored at  $-20^{\circ}\text{C}$ .

**PROBETEC ASSAY.** Specimens were processed and assayed according to the manufacturer's instructions (Becton Dickinson, Sparks, MD). A ProbeTec result was considered high positive if the MOTA score was  $> 10,000$ . Patient specimens with results between 2000 and 9999 were considered low positive and were repeated prior to reporting. Following testing, processed specimens were stored at  $-20^{\circ}\text{C}$ .

**DISCREPANT ANALYSIS.** PACE 2 and ProbeTec results were compared. Results that agreed were considered true positives or true negatives. If the results did not agree, both assays were repeated. If after repeat testing the results agreed, the results were considered true positives or true negatives. If the results did not agree, both the PACE 2 and ProbeTec specimens were tested with the Cobas Amplicor CT/NG assay (Roche, Indianapolis, IN). To remove inhibition from the PACE 2 diluent, samples were washed using the urine wash method following the manufacturer's instructions. The Cobas result was considered the true result.

## RESULTS

- A total of 467 endocervical specimens were submitted for CT/NG testing over a three-month period.
  - 5 (1.1%) specimens were QNS for complete testing.
  - 462 specimens were available for complete testing and resolution of discrepant results.
- After initial testing, a total of 46 (10.0%) specimens were positive for CT (Table 1).
  - 32/46 (69.6%) were positive by both PACE 2 and ProbeTec.
  - 14/46 (30.4%) were positive by ProbeTec only.
  - 0/46 (0%) were positive by PACE 2 only.
- After initial testing a total of 9 (1.9%) specimens were positive for NG (Table 2).
  - 8/9 (88.9%) were positive by both PACE 2 and ProbeTec.
  - 1/9 (11.1%) were positive by ProbeTec only.
  - 0/9 (0%) were positive by PACE 2 only.
- A total of 15 discrepant specimens (14 ProbeTec CT +/PACE 2 CT - and 1 ProbeTec NG +/PACE 2 NG -) were retested using the Cobas Amplicor CT/NG assay. All 15 (100%) results were positive (14 CT+, 1 NG+). The performance characteristics of the PACE 2 and ProbeTec assays are listed in Table 3.
- To determine the reproducibility of positive ProbeTec results, a total of 49 CT positive specimens (24 low positive and 25 high positive) and 32 NG positive specimens (7 low positive and 25 high positive) were retested.
  - A total of 13/24 (54.2%) low positive CT specimens and 4/7 (57.1%) low positive NG specimens repeated positive.
  - A total of 25/25 (100%) high positive CT specimens and 25/25 (100%) high positive NG specimens remained positive after repeat testing.

Table 1. Comparison of ProbeTec and PACE 2 assays for detection of *Chlamydia trachomatis* in endocervical samples.

	No. of specimens:		
		ProbeTec	Total
PACE 2	Positive	32	32
	Negative	14	430
	Total	46	462

Table 2. Comparison of ProbeTec and PACE 2 assays for detection of *Neisseria gonorrhoeae* in endocervical samples.

	No. of specimens:		
		ProbeTec	Total
PACE 2	Positive	8	8
	Negative	1	453
	Total	9	462

Table 3. Performance characteristics for PACE 2 and ProbeTec in detecting *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

Test	Organism	Sensitivity (%)	Specificity (%)	PPV <sup>a</sup> (%)	NPV <sup>b</sup> (%)
PACE 2	CT <sup>c</sup>	69.6	100	100	96.7
	NG <sup>d</sup>	88.9	100	100	99.8
ProbeTec	CT	100	100	100	100
	NG	100	100	100	100

a PPV=positive predictive value  
b NPV=negative predictive value

c CT=*Chlamydia trachomatis*  
d NG=*Neisseria gonorrhoeae*

## SUMMARY AND CONCLUSIONS

- *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are important causes of sexually transmitted disease. Molecular diagnostic techniques have emerged as reliable alternatives to culture. This study was undertaken to evaluate the performance of a nucleic acid amplification assay, ProbeTec, compared with the currently used non-amplified DNA probe assay, PACE 2, for detection of CT and NG in endocervical specimens. The observations of the study included:
    1. Our population of patients had a much higher rate of infection with CT compared to NG.
    2. ProbeTec detected 30.4% and 11.1% more CT and NG confirmed positive specimens, respectively, than PACE 2.
    3. A significant proportion of ProbeTec low positive specimens did not repeat as positive.
    4. All ProbeTec high positive specimens repeated as positive.
  - In conclusion, ProbeTec is more sensitive than PACE 2 for the detection of CT and NG in endocervical specimens. In addition, only low positive ProbeTec results require repeat testing prior to reporting.
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