

## Evaluation of Three Automated Nucleic Acid Amplification Systems for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in First-Void Urine Specimens<sup>∇</sup>

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**A total of 500 first-void urine specimens were tested for the presence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* nucleic acids using ProbeTec ET reagents on a Viper platform (BD Diagnostics, Mississauga, Ontario, Canada), Aptima Combo 2 reagents on a Tigris platform (Gen-Probe, Inc., San Diego, CA), and Abbott RealTime CT/NG reagents on an m2000 platform (Abbott Molecular Diagnostics, Des Plaines, IL). The performance of the three assays for detection of *N. gonorrhoeae* was comparable, but detection of *C. trachomatis* by the three assays showed more variation. All three platforms were suitable for the detection of *C. trachomatis* and *N. gonorrhoeae*, but additional factors, such as maximum daily specimen throughput, are important in evaluating automated systems for *C. trachomatis* and *N. gonorrhoeae* detection in high-volume laboratories.**

*Chlamydia trachomatis* and *Neisseria gonorrhoeae* are common bacterial sexually transmitted infections. These infections may remain asymptomatic, particularly in female patients. Untreated chlamydial and gonococcal infections may have long-term sequelae, including pelvic inflammatory disease, ectopic pregnancy, and sterility. The benefits of diagnosis and treatment are well understood. Since the introduction of nucleic acid amplification tests for the detection of *C. trachomatis* and *N. gonorrhoeae* in genital tract specimens, this approach has become the standard diagnostic method used in most laboratories. Moreover, urine has also been validated as an adequate specimen for diagnostic testing in many studies (4, 7, 11). We report here on the prospective evaluation of three automated systems for the detection of *C. trachomatis* and *N. gonorrhoeae* in urine specimens.

A total of 500 first-void urine specimens were tested using ProbeTec ET reagents on the Viper platform (BD Diagnostics, Mississauga, Ontario, Canada), Aptima Combo 2 reagents on a Tigris platform (Gen-Probe, Inc., San Diego, CA), and Abbott RealTime CT/NG reagents on an m2000 platform (Abbott Molecular Diagnostics, Des Plaines, IL). The m2000 system is an automated magnetic sample preparation platform combined with homogeneous real-time multiplexed PCR for both *C. trachomatis* and *N. gonorrhoeae* (9). All specimens were tested according to the manufacturer's instructions.

Each of the assays is designed to detect different targets. The primer sequences are proprietary, but the regions targeted are known. The Abbott RealTime CT/NG assays target the cryptic plasmid of *C. trachomatis* and the opacity gene of *N. gonorrhoeae* (9), the ProbeTec ET assays target the cryptic plasmid

of *C. trachomatis* and the pilin-inverting gene of *N. gonorrhoeae* (11), while the Gen-Probe Aptima Combo 2 detects targets in the 23S rRNA of *C. trachomatis* and the 16S rRNA of *N. gonorrhoeae* (3).

In order to increase the ability of the study to detect differences in performance between the three assay systems, an additional number of specimens positive for either *C. trachomatis* or *N. gonorrhoeae* were included among the 500 specimens. This approach allows the determination of sensitivity and specificity but precludes the determination of positive and negative predictive values, which are dependent upon the prevalence in the population studied.

Specimens testing positive for *C. trachomatis* in the ProbeTec ET assay were retested by using the same extraction, while specimens that tested positive for *N. gonorrhoeae* were reextracted and then retested. All specimens that gave positive results in the Aptima Combo 2 assay were retested using the individual Aptima CT or Aptima GC assay as appropriate. The interpretation of the results from the Abbott assay was performed according to the CE (Conformité Européenne) package insert. In this protocol, a minimum of one replicate of the negative control and two replicates of the cutoff control are required with each run. For each analyte assayed (*C. trachomatis* or *N. gonorrhoeae*) the mean cycle number of the cutoff controls is calculated, and then a predetermined number of cycles is added to this mean value to generate the cutoff decision cycle (CO). If the assayed sample generates a cycle number less than or equal to the CO, a positive interpretation and a numerical result  $\geq 0$  is reported. The numerical result corresponds to the difference in cycle number between the CO and the sample cycle number. A sample that does not generate a cycle number is reported as negative. A sample that exceeds the CO but generates a valid cycle number with an interpretation of negative is required to be retested. If the retest interpretation is positive, the sample is reported as positive. If the retest interpretation is negative and the sample has a reported cycle number exceeding the CO, the sample is reported as posi-

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TABLE 1. Comparative performance of three systems for detection of *N. gonorrhoeae* in urine

Parameter	BD ProbeTec ET	Abbott m2000	Gen-Probe Aptima Combo 2
No. true positive	23	24	24
No. false positive	0	0	0
No. false negative	1	0	0
No. true negative	476	476	476
% Sensitivity	95.8	100	100
% Specificity	100	100	100

tive. If the retest interpretation is negative and no amplification curve is observed, the sample is reported as negative.

Specimens that gave discrepant results among the three commercial assays were retested with in-house real-time PCR assays. For *C. trachomatis* the assay target was pCHL1 (8), and for *N. gonorrhoeae* the target was *cppB* (5). The results for each assay were then classified as true positive, false positive, false negative, and true negative. If a result from one assay was negative and the other two assays were positive and confirmed positive by the in-house assay, the negative result was designated as a false negative. Similarly, a positive result from one assay was designated as a false positive if the other two assays were negative and confirmed negative by the in-house assay.

The performance of the three assays for detection of *N. gonorrhoeae* was comparable (Table 1). The ProbeTec ET assay failed to detect one specimen that was positive by both the Abbott RealTime and Aptima Combo 2 assays. The detection of *C. trachomatis* by the three assays showed more variation (Table 2). The Aptima Combo 2 assay was the most sensitive, and the Abbott RealTime CT/NG assay was the least sensitive. However, these assays showed greater specificity than the ProbeTec assay. Similar findings have been reported previously (1).

Only the Aptima Combo 2 used confirmatory assays that used different targets than the primary assay, as recommended in the Centers for Disease Control and Prevention guidelines for detection of *C. trachomatis* and *N. gonorrhoeae* (6) and for medicolegal testing purposes by the Expert Working Group on Canadian Guidelines for Sexually Transmitted Infections (2).

Both the ProbeTec ET and Gen-Probe Aptima Combo 2 assays detect the mutant strain of *C. trachomatis* recently described in Sweden (10). The Abbott RealTime CT/NG reagents studied did not detect the variant strain. An additional primer pair and a probe have been developed and will be available commercially (5a).

Important differences between the assays in maximum daily throughput were observed. The Gen-Probe Tigris system had the highest throughput and was the least labor-intensive. After initial maintenance and quality checks at the start of the work shift, the Tigris system required only periodic attention for loading and unloading racks of specimens. The BD Viper system required more hands-on time, due largely to specimen processing before loading onto the Viper instrument and to the requirement for manual transfer of sealed microwell plates to the BD ProbeTec ET incubator-reader. This system was the most labor-intensive of those studied. The Abbott m2000 system had the lowest throughput, estimated to be 186 specimens per 8-h shift (9), but required the least hands-on time. Speci-

TABLE 2. Comparative performance of three systems for detection of *C. trachomatis* in urine

Parameter	BD ProbeTec ET	Abbott m2000	Gen-Probe Aptima Combo 2
No. true positive	96	95	97
No. false positive	2	0	0
No. false negative	2	3	1
No. true negative	400	402	402
% Sensitivity	97.95	96.94	98.98
% Specificity	99.5	100	100

men turnaround times are affected very much by specimen delivery schedules and lab operating hours. We did not calculate turnaround times in this study.

Since the introduction of urine-based nucleic acid amplification tests in our laboratory in 2000, test volumes have risen to over 50,000 per annum, of which 8.7% were positive for *C. trachomatis* and 2.1% were positive for *N. gonorrhoeae* in 2007. Each of the assay systems studied produced acceptable results, but additional factors, other than analytical sensitivity and specificity, are important in evaluating automated systems for *C. trachomatis* and *N. gonorrhoeae* detection in high-volume laboratories.

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