JOURNAL OF CLINICAL MICROBIOLOGY, July 2006, p. 2619–2621 Vol. 44, No. 7
0095-1137/06/$08.00+0 doi:10.1128/JCM.00476-06
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Comparison of the Gen-Probe APTIMA Combo 2 Assay to the AMPLICOR CT/NG Assay for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Urine Samples from Australian Men and Women

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Received 6 March 2006/Returned for modification 21 April 2006/Accepted 2 May 2006

The performance of the APTIMA Combo 2 assay (AC2) for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in urine samples was compared to that of the AMPLICOR CT/NG assay (AMP). The AC2 performance was superior to that of AMP for both organisms in this study.

Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections may be performed using various approved genital specimens such as endocervical swabs, vaginal swabs, male urethral swabs, and urine specimens from both sexes. Detection of *C. trachomatis* and *N. gonorrhoeae* in urine specimens is easy, rapid, and noninvasive and is often the patients' preferred collection method. Our laboratory routinely tests over 1,100 specimens per week for *C. trachomatis* and/or *N. gonorrhoeae* infections.

First-catch urine specimens submitted for routine *C. trachomatis* and *N. gonorrhoeae* detection from 2,973 patients were included in this study. All 2,973 specimens were tested for *C. trachomatis*. *N. gonorrhoeae* testing was performed on 1,535 specimens. Specimens were processed according to the manufacturer's instructions (7, 16). All samples for AMPLICOR CT/NG assay (AMP) testing were processed within 24 h of collection. APTIMA Combo 2 assay (AC2) testing was completed within 48 h. Results positive for *N. gonorrhoeae* by AMP were confirmed with the *cppB* assay on the Roche LightCycler to exclude false-positive results attributable to nonpathogenic *Neisseria* spp. (12, 20). Samples with discordant results by AMP and AC2 were repeated with each assay and additionally tested with APTIMA *C. trachomatis* and *N. gonorrhoeae* assays (ACT and AGC, respectively). These individual assays detect different target sequences using primers different from those used in AMP and AC2. Samples were considered true positives according to Australian NPAAC guidelines (15), i.e., if they were positive in two independent assays utilizing different primers. It is possible that samples that were positive by only one assay may be true positives because of differences in analytical sensitivities.

ACT and AGC are nucleic acid amplification tests for the detection of *C. trachomatis* and *N. gonorrhoeae* rRNA genes, respectively. ACT and AGC are suitable and recommended for the confirmation of positive results from AC2 and AMPLICOR because they target different nucleic acid sequences using different primers (2). In this laboratory, the *cppB* assay on the Roche LightCycler was routinely used to confirm all results that were positive for *N. gonorrhoeae* by AMP. This assay has been shown to be adequate for confirming *N. gonorrhoeae*-positive results by AMP with a sensitivity above 98% (19). However, recent publications have shown that this target may not be suitable as a confirmatory assay for all populations (1, 3).

Of the 2,973 samples tested for *C. trachomatis* using both AMP and AC2 (56.5% female and 43.5% male), 8 samples had an uncertain status and were removed from the final analysis. These samples were initially discordant, and their status was unable to be satisfactorily resolved by repeat testing by both assays due to sample volume limitations and/or variations in the results obtained (results changed from positive to negative and back again, which may represent sampling issues and the limits of detection of the assays). The results were reported as equivocal, requiring a repeat sample to be submitted. Two of these samples were posttreatment specimens. Of the 2,965 samples in the final analysis, 2,644 (89.2%) were negative (considered true negatives) and 285 (9.6%) were positive (considered true positives) by both AC2 and AMP. A total of 36 (1.2%) samples were positive by AC2 and negative by AMP for *C. trachomatis* and were therefore considered discordant samples. These discordant samples were retested in each assay and with the ACT. Of the 36 discordant samples, 19 were positive by AC2, repeatedly negative by AMP, and positive by ACT; these samples were considered true positives and therefore false negatives by AMP. Eleven of the discordant samples were positive by AC2, positive by AMP upon repeat testing, and positive by ACT; these samples were considered true positives and therefore initially false negatives by AMP. The last six discordant samples were positive by AC2, negative by AMP, and negative by ACT; these samples were considered true negatives and therefore false positives by AC2. In total, 9.9% and 11.6% of samples from women and men, respectively, were considered positive. A summary of the resolved data are shown in Table 1.

Of the 1,535 samples tested for *N. gonorrhoeae* with both AMP and AC2, 2 samples were initially discordant and had uncertain status. Insufficient sample volume was available for

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TABLE 1. Resolved *C. trachomatis* results[a]

Test and result	No. of samples with ACT result that was:		PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
	Positive	Negative				
AC2						
Positive	315	6	98.1		100.0	99.8
Negative	0	2,644		100.0		
AMP						
Positive	285	0	100.0		90.5	100
Negative	30	2,650		98.9		

[a] Prevalence, 10.6%. NPV, negative predictive value; PPV, positive predictive value.

TABLE 2. Resolved *N. gonorrhoeae* results[a]

Test and result	No. of samples with ACT result that was:		PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
	Positive	Negative				
AC2						
Positive	45	0	100.0		100.0	
Negative	0	1,488		100		100.0
AMP						
Positive	39	3	92.9		86.7	
Negative	6	1,485		99.6		99.8

[a] Prevalence, 2.9%. PPV, positive predictive value; NPV, negative predictive value.

repeat testing, and these samples were removed from the final analysis. Of the 1,533 samples in the final analysis, 1,485 (96.9%) were negative (considered true negatives) and 39 (2.5%) were positive (considered true positives) using AC2, AMP, and the *cppB* assay. A total of nine (0.6%) samples were discordant. These discordant samples were retested by each assay and with AGC and the *cppB* assay for resolution. Of the nine discordant samples, five were positive by AC2, negative by AMP, and positive by AGC; these samples were considered true positives and therefore false negatives by AMP. One of the discordant samples was positive by AC2, positive by the AMP assay upon repeat, and positive by AGC; this sample was considered a true positive and therefore initially false negative by AMP. These six samples were not tested with the *cppB* assay. The last three discordant samples were negative by AC2, positive by AMP, and negative by the *cppB* assay; these samples were considered true negatives and therefore false positives by AMP. In total, 1.8% and 3.3% of samples from women and men, respectively, were considered positive. A summary of the resolved data are shown in Table 2.

The higher clinical sensitivity of AC2 may be due to its higher analytical sensitivity. A direct comparison showed that the AC2 analytical sensitivity for *C. trachomatis* detection was approximately 100-fold higher than that of AMP (0.008 versus 0.5 elementary bodies per assay) (9). The higher sensitivity of AC2 for urine specimens may reflect the target capture method preceding transcription-mediated amplification, which allows the separation of the target sequences from the urine matrix, limiting the effects of inhibitors on the amplification step (4, 7). Although an internal control was not routinely performed with AMP, some initially negative specimens were positive upon repeat testing (samples were stored at 4°C overnight prior to retesting). This suggests that inhibitors may have been present initially. AMP has been shown to be less sensitive than AC2 for testing of urine specimens in two previous studies (5, 6). Amplification inhibition with AMP appears to be particularly problematic with urine specimens from women (11), and some studies have recommended not using AMP for the detection of *N. gonorrhoeae* in urine from women due to the assay's low sensitivity with this type of specimen (12, 14).

AMP yielded three false positives for *N. gonorrhoeae* detection (of a total of 45 true *N. gonorrhoeae* positives, i.e., 6.6% false positives) since these samples were negative by AC2, AGC, and *cppB* assay while positive by AMP. AMP has been shown to detect nonpathogenic *Neisseria* spp. (12, 20), hence the recommendation to confirm any *N. gonorrhoeae*-positive results by AMP with another assay (20). The lack of accuracy of AMP for *N. gonorrhoeae* detection and the need to confirm positive results make this assay unsuitable for *N. gonorrhoeae* detection, especially in populations with low *N. gonorrhoeae* prevalence. AC2, on the other hand, yielded no false positives or false negatives for *N. gonorrhoeae* detection and had a sensitivity and specificity of 100% each. AC2 should not require a confirmation of *N. gonorrhoeae*-positive samples (8) because it does not cross-react with other *Neisseria* spp. and generally yields a very high sensitivity for *N. gonorrhoeae* detection in urine specimens (6, 8, 13, 18). Therefore, these attributes make AC2 a good assay for *N. gonorrhoeae* detection in the clinical laboratory setting.

In the present study, six samples that tested positive for *C. trachomatis* by AC2 could not be confirmed by AMP and by ACT and were therefore considered false positives by AC2. Clinical investigations of these six patients revealed that two patients had been treated for chlamydial infection and that two patients had partners with chlamydial infection. This suggests that these samples may have in fact been true positives and were correctly detected as such by AC2, consistent with the higher analytical sensitivity of this assay. Thus, the true specificity of AC2 for *C. trachomatis* detection may be even greater than that calculated in this study. Other studies have also reported that the calculated specificity of a new assay can be artificially lower than its true value because the new assay is compared with older standard assays with inferior performance (6, 13, 17).

AC2 is an FDA-cleared assay for the detection of *C. trachomatis* and *N. gonorrhoeae* in urine specimens from men and women. In our hands, AC2 had a very high sensitivity (100% for both *C. trachomatis* and *N. gonorrhoeae* detection) and very high specificity (>99%). These data question the current requirement for alternate confirmatory testing of positive samples (10) when this method is used. AC2 showed a good reproducibility and, unlike AMP, appeared to be unaffected by inhibitory substances in urine specimens. It is recommended that users of AMP routinely include an internal control in light of the number of samples that tested positive upon repeat testing. Because of its improved performance over AMP, AC2 should be recommended for the routine noninvasive detection of *C. trachomatis* and *N. gonorrhoeae* in male and female urine

specimens in the clinical laboratory, an approach particularly suitable for *C. trachomatis* and/or *N. gonorrhoeae* screening campaigns.

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