C-174. Comparison of Gen-Probe APTIMA® Combo 2 (AC2) Assay for *C. trachomatis* (CT) and *N. gonorrhoeae* (GC) with ProbeTec and LCx on Urine and Swab Specimens from Women Using a Rotating Standard

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INTRODUCTION

Determination of the appropriate standard for measuring the performance of nucleic acid amplification tests (NAAT) for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) in genitourinary specimens has proven to be difficult. When NAATs were first developed for CT, culture was the gold standard for diagnosing this organism. Based on knowledge of the analytical sensitivity of NAATs plus the fact that it was known that culture was not optimally sensitive, it was reasonably clear that the new tests were more sensitive than the gold standard culture. These studies showed that the use of culture alone as a reference standard resulted in significant underestimates of the specificity for the new assays (Schachter, 1995). To address this problem, investigators applied an alternate target amplification assay to the putative false positive results only (discrepancy analysis). Unfortunately, this approach is biased towards over estimating sensitivity and may also over estimate specificity (Hadgu). Though the extent of the bias appears minimal, the resulting controversy has resulted in a lack of acceptance of this approach for determining the performance characteristics of new NAATs (Green, Miller, Schachter, 1998; McAdam). Now that several NAATs have been cleared for commercial use, these tests could be used to assess the performance of newer NAAT s for the detection of CT and GC without the use of culture. Johnson et al. has shown that a single NAAT standard substituted for culture significantly improved performance estimates (Johnson, 2000).

In this study, we examined the performance of three different NAATs for testing swab and urine specimens for CT and GC in women. The performance of each assay was measured against the other two assays in rotation. A possible problem with comparing sensitivity and specificity between the three assays based on such an analysis is the potential for unpredictable bias because each of the assays being compared is used as part of the "gold standard" for the determination of the performance of the other assays. Therefore, in order to determine the robustness of the rotating standard comparison we compared the APTIMA Combo 2 assay to LCx using ProbeTec only as the "gold standard" and, in a second analysis we compared APTIMA Combo 2 to ProbeTec using LCx only as the "gold standard."

MATERIALS AND METHODS

Study Site

Specimens were collected from symptomatic and asymptomatic women attending the City of New Orleans STD clinic. The informed consent was obtained from each patient and the study was approved by the LSU IRB.

Study Design

Urine and endocervical swab specimens were tested by three nucleic acid amplification assays (NAATs) for CT and GC. Sensitivity and specificity were calculated to summarize performance characteristics for each organism and specimen type.

Specimen collection procedures

Women provided at least 25 mL of first catch urine (FCU and four endocervical swab specimens for testing by the Combo 2, LCx, and the ProbeTec assays. The FCU specimen was collected before the swab specimens. The collection order of the swabs was determined by a randomization protocol. All specimens were collected, stored, and transported to the laboratory according to each assay manufacturer's instructions. Specimens were excluded if the patient had urinated one hour prior to providing the specimen, or had taken antibiotic medication within the previous 21 days; or if collection, storage, or transport requirements were not met.

Description of assays

The APTIMA Combo 2 Assay (Gen-Probe Incorporated, San Diego, California) is a target-amplified nucleic acid probe test that employs target capture for the *in vitro* qualitative detection and differentiation of ribosomal RNA (rRNA) from CT and GC in endocervical and male urethral swab specimens, and in female and male urine specimens. The TMA products are detected using the LEADER HC+ Luminometer.

The LCx *Neisseria gonorrhoeae* assay and the LCx *Chlamydia trachomatis* assay (Abbott Laboratories, Abbott Park, Illinois) use the ligase chain reaction to amplify plasmid DNA. The LCR reaction product is detected by the Abbott LCx Analyzer.

The BDProbeTec™ ET System (ProbeTec) (BD Diagnostic Systems, Sparks, MD) uses strand displacement amplification technology and fluorescent detector probes to detect CT and GC DNA from samples. An inhibition control is run for each sample.

Determination of infected patient standard

Rotating standard: On a rotating basis, the swab and urine CT and GC results of each of three NAATs were compared to an infected patient standard based on the other two NAATs for each organism in separate analyses. The infected patient standard was defined as any two positive specimens by either of the two comparator NAATs. If only one of the comparator assays was positive, the results were considered inconclusive and were not in included in the analysis.

Single assay standards: In the first analysis, LCx results were used as the standard. If either the swab or urine specimen was positive, the patient was defined as infected. Sensitivity and specificity were then calculated based on this standard for the Combo 2 and ProbeTec assays and the two were compared. In a second analysis, ProbeTec results were used as the standard using the same definition for the infected patient. In this analysis, the performance of Combo 2 was compared to LCx and separate analyses were performed for both CT and GC.

RESULTS

A total of 290 patients were enrolled. Of those enrolled, 271 (93%) subjects had a complete set of CT tests and 269 (93%) had a complete set of GC tests. Among the 273 women included in the analysis, 94% were African-American. Mean age was 25. Overall, 76% complained of symptoms. The presence or absence of symptoms did not influence the outcome so cases are combined in the data presented below. Twelve percent of women attended the clinic because of contact to a man with CT and 13% because of exposure to GC.

Results of the analyses are summarized in the following tables:

Table 1. Comparison of All Three NAATs Based on the Rotating Standard*

	Sensitivity		Specificity	
	Swab	Urine	Swab	Urine
C. trachomatis				
Combo 2	91.5	94.9	94.6	99.0
ProbeTec	81.5	78.5	100	99.5
LCx	80.3	74.2	100	100
N. gonorrhoeae				
Combo 2	100	100	99.0	100
ProbeTec	96.4	94.5	99.5	100
LCx	92.7	89.1	98.6	100

*Patients meeting the definitions for infection were as follows: Combo 2 CT analysis-59, ProbeTec CT analysis-66, LCx CT analysis-65. For each of the three GC analyses there were 55 infected patients. The number of inconclusive results for each analysis were as follows: Combo 2 CT analysis 7, ProbeTec CT analysis 11, LCx CT analysis 11, Combo 2 GC analysis 4, ProbeTec GC analysis 3, LCx GC analysis 5.

Table 2. Combo 2 Compared to ProbeTec Based on an LCx Single Test Standard*

	Sensitivity		Specificity	
	Swab	Urine	Swab	Urine
C. trachomatis				
Combo 2	91.8	93.4	93.8	97.1
ProbeTec	85.2	82.0	99.5	98.1
N. gonorrhoeae				
Combo 2	94.7	94.7	98.6	99.5
ProbeTec	91.2	89.5	99.1	99.5

*For CT, 61 women were positive by either the LCx swab or the LCx urine test and were classified as infected. For GC, 57 women met the infected patient definition for this analysis.

Table 3. Combo 2 Compared to LCx Based on a ProbeTec Single Test Standard*

	Sensitivity		Specificity	
	Swab	Urine	Swab	Urine
C. trachomatis				
Combo 2	87.5	93.8	93.7	98.6
LCR	81.3	76.6	99.0	100
N. gonorrhoeae				
Combo 2	98.2	98.2	99.1	100
LCR	91.1	87.5	98.6	100

*For CT, 64 women were positive by either the ProbeTec swab or the ProbeTec urine test and were classified as infected. For GC, 56 women met the infected patient definition for this analysis.

SUMMARY AND CONCLUSIONS

- Combo 2 was consistently more sensitive than LCx and ProbeTec for both CT and GC though the differences were greatest for CT. It can be argued that comparisons based on the rotating standard are subject to bias because calculation of sensitivity and specificity for each of the assays being compared requires use of the other two assays as part of the rotating gold standard. In order to determine the robustness of the rotating standard estimates, we compared Combo 2 to LCx in one analysis using ProbeTec as a single assay gold standard. In a second analysis, we compared Combo 2 to ProbeTec using LCx as the standard. In both cases, Combo 2 was more sensitive than the other two assays, thus providing validation of the rotating standard as it was applied to this data base. A previously reported analysis of data from another study showed similar results for Combo 2, LCx and the Roche Amplicor assay (Martin).
- Specificity of Combo 2 was lower for CT than the other two assays for endocervical swab specimens. Of the ten false positive swab specimens, six immediately followed a clearly infected case based on multiple positive specimens. This suggests that these specimens may have been cross-contaminated. In general, lower specificity for Combo 2 could be due to something intrinsic in the assay that results in false positives or it could be that this assay identifies truly infected patients. These patients may have bacterial loads that are too low to be detected by LCx and/or ProbeTec, or the specimens have inhibitors that do not interfere with the Combo 2 assay because of the target capture methodology. The fact that Combo 2 is consistently more sensitive than the other NAATs is in accord with the latter explanation. Confirmation of this hypothesis would require "discrepant analysis."
- There was little difference in the sensitivity between swab and urine specimens for the detection of CT in this study, though for both the LCx and ProbeTec assays swab specimens had a modest but consistent advantage. Of interest is the fact that urine specimens were as sensitive or even more sensitive than swab specimens by the Combo 2 test. As urine specimens are thought to be more subject to inhibitors than swab specimens this observation likely reflects the efficacy of the target capture step in the Combo 2 assay.

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