C-175. HEAD-TO-HEAD STUDY COMPARING PERFORMANCE OF THE GEN-PROBE APTIMA® Combo 2, ABBOTT LCX® PROBE, BECTON DICKINSON BDProbeTec™ ET, AND ROCHE AMPLICOR® NUCLEIC ACID AMPLIFICATION ASSAYS IN DETECTING *CHLAMYDIA TRACHOMATIS* FROM FIRST CATCH URINE

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REVISED ABSTRACT

APTIMA Combo 2 (Gen-Probe Incorporated) performance in detecting *Chlamydia trachomatis* (CT) from first catch urine (FCU) was compared to the LCx Probe (Abbott Laboratories), BDProbeTec ET (Becton Dickinson Company), and AMPLICOR (Roche Molecular Diagnostics) nucleic acid amplification systems. FCU specimens were collected from 237 female and 266 male patients attending sexually transmitted disease and juvenile detention center clinics. Specimens were collected, transported, stored and tested according to the manufacturer's recommendations for each assay. Test performance was evaluated on the basis of infected patient status, defined as having any two or more assay results positive. The overall prevalence of CT was 7.8%. There were 464 specimens that were negative in at least two assays. There were 37 specimens that were positive in at least two assays and 29 specimens that were positive in all four assays. When compared to infected patient status, Combo 2 sensitivity and specificity was 100%. When compared to infected patient status, all other manufacturer's specificities were 100%. However, sensitivities when compared to infected patient status for female FCU varied from 76% in the LCx assay, to 86% in the BDProbeTec ET and the AMPLICOR assays. Sensitivities when compared to infected patient status for male FCU varied from 83% in the LCx assays and AMPLICOR assays to 89% in the BDProbeTec ET assay. There were 90 inhibited specimens; 89 occurred in the BDProbeTec ET assay and one occurred in the AMPLICOR assay. Two specimens that were positive by Combo 2 but negative in the other assays were retested by a transcription mediated amplification (TMA) assay targeting a different rRNA molecule. These TMA alternate amplification results were positive which suggests that the original Combo 2 positive results were correct and the comparator assay results were false negative. Two specimens that were positive by Combo 2 were inhibitory in the BDProbeTec ET assay. Overall, Combo 2 compared equally well to the three other assays in specificity. However, Combo 2 outperformed the three other assays in sensitivity.

INTRODUCTION

Sexually transmitted disease caused by *Chlamydia trachomatis* (CT) continues to be a serious threat to infected individuals and their partners. The number of reported cases continues to rise making the need for early, accurate diagnosis and adequate treatment even more critical. Nucleic acid amplification tests (NAATs) based on the polymerase chain reaction (PCR), ligase chain reaction (LCR), transcription mediated amplification (TMA), and strand displacement amplification (SDA) assays have been developed and are now in routine use in many laboratories around the world. The performance of these tests using both swab and first catch urine (FCU) specimens has been documented in numerous clinical studies. The ability to screen adolescents, the capability of testing noninvasive specimens such as urine and the ability to acquire specimens in non-traditional venues has been reported to be an important intervention effort. These studies tell us that the health of certain individuals and populations could be improved and that the overall cost to society could be reduced through early detection and treatment of cases identified through the use of NAAT. The newest United States Food and Drug Administration (FDA) -cleared NAAT is the APTIMA Combo 2 assay (Combo 2) which was cleared in May, 2001 and is available for routine clinical use. Recent studies have compared some NAATs head to head. The results of the study reported here compares Combo 2 performance in detecting CT from FCU to the LCx Probe (Abbott Laboratories), BDProbeTec ET (Becton Dickinson Company), and AMPLICOR (Roche Molecular Diagnostics) nucleic acid amplification systems. We believe this to be the first reported clinical study comparing the performance of all four FDA -cleared NAAT technologies using the same urine specimen.

METHODS

A total of 503 FCU specimens were collected from 237 female and 266 male patients attending sexually transmitted disease and juvenile detention center clinics. Specimens were collected, transported, stored and tested according to the manufacturer's recommendations for each assay. Test performance was evaluated on the basis of infected patient status, defined as having any two or more assay results positive. Samples positive in only the Combo 2 assay were tested in a TMA system targeting alternate sequences. Any unresolved inhibited specimens were noted, but excluded from the comparison, unless positive in three other assays.

Combo 2 (Gen-Probe, Incorporated, San Diego, California) utilizes the TMA technology, which targets rRNA. Combo 2 incorporates the "target capture" technique, which uses hybridization and magnetic particles to isolate the target sequences and separate them from the specimen matrix, including amplification inhibitors that may be present. Combo 2 is a multiplex second-generation TMA test in which specific regions of the CT and N. gonorrhoeae (GC) rRNA are isolated and amplified using a separate capture oligomer and a unique set of primers for each target. Simultaneous detection and differentiation of the CT and GC amplicons is achieved using the Dual Kinetic Assay (DKA) method. DKA uses single-stranded DNA probes labeled with two different types of acridinium ester (AE) molecules. Differences in the kinetic profiles of the labeled probes specific for CT and GC differentiate the results for the two pathogens.

RESULTS

The overall prevalence of CT was 7.8% (39/503). There were 464 specimens that were negative in at least two assays. There were 37 specimens that were positive in at least two assays and 29 specimens that were positive in all four assays. When compared to infected patient status, Combo 2 sensitivity (39/39) and specificity (464/464) was 100%. When compared to infected patient status, all other manufacturer's specificities were 100%. However, sensitivities when compared to infected patient status for female FCU varied from 76% (16/21) in the LCx assay, to 86% (18/21) in the BDProbeTec ET and the AMPLICOR assays. Sensitivities when compared to infected patient status for male FCU varied from 83% (15/18) in the LCx assays and AMPLICOR assays to 89% (16/18) in the BDProbeTec ET assay. There were 90 inhibited specimens; 89 occurred in the BDProbeTec ET assay and one occurred in the AMPLICOR assay. Two specimens that were positive by Combo 2 but negative in the other assays were retested by the TMA alternate amplification assay and were found to be positive, indicating the presence of CT rRNA. Two specimens that were positive by Combo 2 were inhibitory in the BDProbeTec ET assay.

Table 1 - Comparison Female Urine specimen tested in the APTIMA Combo 2 Assay and Roche Monitor Amplicor Chlamydia Assay

		Positive[a]	Negative[b]	Inhibitory	Total
Combo 2	Positive	18	3		21
	Negative	0	215	1	216
	Total	18	218	1	237

[a] One specimen was initially invalid in the Combo 2 Assay, value was too high. Upon repeat sample was Chlamydia Positive.
[b] One specimen was CT positive in Combo assay only. One specimen was CT positive by LCx and Combo.

Table 2 - Comparison of APTIMA Combo 2 to Becton Dickinson ProbeTec with Female Urine Specimens for Chlamydia

		BD ProbeTec			
		Positive[a]	Negative[c]	Inhibitory[d]	Total
Combo 2	Positive	18	2	1	21
	Negative	0	191	25	216
	Total	18	193	26	237

[a] One specimen was initially invalid in the Combo 2 Assay, value was too high. Upon repeat testing specimen was CT Positive.
[c] One Positive specimen was CT positive by Combo only. One was CT positive by Roche and Combo. One Negative Specimen was found to be Equivocal by both assays, but upon retest was negative.
[d] One specimen found CT positive by Combo, Roche, and LCx. 12% of Female urine specimens were inhibitory.

Table 3 - Comparison of APTIMA Combo 2 to Abbott LCx Chlamydia in Female Urine Specimens.

		LCx Results		
		Positive[a]	Negative[e]	Total
Combo 2	Positive	16	5	21
	Negative	0	216	216
	Total	16	221	237

[a] One specimen was initially invalid in the Combo 2 Assay, value was too high. Upon repeat sample was Chlamydia Positive.
[e] One Specimen was found to be Equivocal by both assays, retest was negative by both.

Table 4 - Comparison of Male urine specimens with APTIMA Combo 2 and Roche Amplicor Assays For Chlamydia

		Roche Amplicor			
		Positive	Negative	Equivocal[f]	Total
Combo 2	Positive	15	2	1	18
	Negative	0	248		248
	Total	15	250	1	266

[f] One specimen was equivocal, negative upon repeat

Table 5 - Comparison of Male Urine speicmen in the APTIMA Combo 2 and Becton-Dickinson ProbeTec Chlamydia Assay.

		BDProbeTec Results			
		Positive	Negative	Inhibitory[g]	Total
Combo 2	Positive	16	1	1	18
	Negative	0	186	62	248
	Total	16	187	63	266

[g] One specimen CT positive by Combo, Roche and LCx was inhibitory by BD. 62 specimens were initialy and repeat test inhibitory with BD. 31% of urine specimens were inhibitory.

Table 6 - Comparison of Male Urine Specimen with APTIMA Combo 2 and LCx Chlamydia Assays

		LCx Results		
		Postive	Negative[h]	Total
Combo 2	Positive	15	3	18
	Negative	0	248	248
	Total	15	251	266

[h] One specimen was CT positive by Combo, BD and Roche and was negative by Abbott.

COMBO VS.	Sensitivity	Specificity
Roche Male	83%	100%
BD Male	89%	100%
LCx Male	83%	100%
Roche Female	86%	100%
BD Female	86%	100%
LCx Female	76%	100%

Pos. Specimens	Combo 2	Roche	BD	LCx
1 female*	+	N	N	N
2	+	N	Inhibitory	+
3	+	+	N	N
4 & 5	+	+	+	N
6	+	N	+	N
7 male*	+	N	N	N
8	+	N	+	N
9	+	Equ	+	+
10 male	+	+	Inhibitory	+
11	+	+	+	N
12-39 male/female	+	+	+	+

* Only Positive with Combo test, alternate amplification test confirmed positive.

CONCLUSION

Overall, Combo 2 compared equally well to the three other assays in specificity. However, Combo 2 out-performed the three other assays in sensitivity. The TMA alternate amplification results, which were positive, suggest that the original Combo 2 positive results were correct and the comparator assay results were false negative. The development of newer, more sensitive technologies for the detection of STDs has potential benefits for individual patients and public health screening programs. False negative results can lead to adverse medical consequences for the patient and the spread of infection to others. Specificity is an equally important issue with STDs, because of the potential for adverse social, psychological and personal consequences with a false positive result. As new, more sensitive technologies have been developed, they have appeared to have a high "false positive" rate compared with established technologies. There has been concern about the specificity of amplification assays because of the possibility of carryover contamination and cross-reactivity with closely related organisms. In a previous study (unpublished Combo 2 FDA clinical trial data), the alternate amplification TMA confirmatory *CT* assay gave positive results for 97% of concordant positive specimens. We therefore regard the results of the confirmatory TMA assays as reliable in resolving discrepancies. The results of the alternate TMA amplification confirmatory testing also make the case for the availability of alternate amplification as a confirmatory assay for NAATs. The detection of a greater number of true infections is the greatest benefit of the rapidly developing NAAT technology, with benefits to both individual health and to public health screening programs by decreasing the reservoir of infection within the population. Enhanced assay sensitivity using urine specimens also permits the increased use of urine specimens. Until now, NAAT performance using FCU specimens have suffered from the effect of inhibitors. Target capture specimen processing has significantly enhanced the performance of the Combo 2 assay, especially for urine specimens. One of the most important aspects of this study is the high sensitivity of the Combo 2 assay with urine specimens when compared to the sensitivities of the other NAATs. It is particularly important for public health screening programs to obtain reliable results on as many urine specimens as possible during the initial specimen acquisition since these programs rely on field outreach efforts that screen in venues where specimen acquisition is difficult or costly.