Analytical Sensitivity Comparison of *Chlamydia trachomatis* (CT) Nucleic Acid Amplification Assays

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INTRODUCTION

Several nucleic acid amplification tests (NAATs) are available for the detection of *Chlamydia trachomatis* (CT) from urine specimens. In many clinical studies NAATs have been shown to have superior sensitivity performance over non-amplification methods. One important factor in selection of the best NAAT is the sensitivity of one assay when compared to another assay. This study was designed to compare the relative analytical sensitivity of the new APTIMA Combo 2 assay (Combo 2) (Gen-Probe Incorporated, San Diego, California) to the LCx Probe (Abbott Laboratories), Becton-Dickinson Probe Tec ET (Becton Dickinson Company), and COBAS Amplicor (Roche Molecular Diagnostics) nucleic acid amplification systems. The Combo 2 assay utilizes Transcription-Mediated Amplification (TMA) technology. The TMA system targets rRNA, which is present in abundance in all bacterial cells, to increase the sensitivity of the assay. Combo 2 also incorporates a "target capture" technique, which uses hybridization and magnetic particles to isolate the target sequences and separate them from the specimen matrix, which may contain amplification inhibitors. The target capture specimen preparation method does not require mechanical manipulation such as centrifugation and decanting of the specimen that is performed in the other NAATs. There has been a concern that this manipulation in the other assays could lead to the loss of elementary bodies (EB) containing CT nucleic acid target which could reduce the sensitivity of these assays.

METHODS

Three aliquots of KOVA Trol (reconstituted preparation of freeze dried human urine) were prepared to contain CT EB at levels of 100, 10, and 1 EB per ml. The CT EB were grown in McCoy cell monolayers and harvested to yield stocks. The stocks were diluted in phosphate buffered saline. The titer of the EB was determined by infecting fresh monolayers with varying dilutions, followed by DFA staining. Ten replicates of each level were assayed in each technology. The 100 EB per ml processed sample was then further diluted 1:10, 1:100, and 1:1000 in the appropriate sample buffer for each assay and tested in 10 replicates.

Fig. 1 Target Capture Specimen processing technique used in the APTIMA Combo 2 Assay

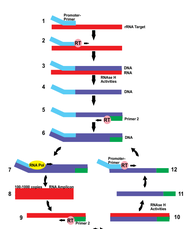

Fig. 2 Transcription-Mediated Amplification (TMA)

RESULTS

Combo 2 detected CT in all 10 of 10 replicates from the 100 EB/ml, 10 EB/ml and 1 EB/ml levels of EB concentrations. COBAS detected CT in 10 of 10 replicates from the 100 EB/ml level, 9 of 10 from the 10 EB/ml level and 2 of 10 at the 1 EB/ml level. LCx detected CT in 10 of 10 replicates from the 100 EB/ml level, 5 of 10 from the 10 EB/ml level and 0 of 10 at the 1 EB/ml level. ProbeTec detected CT in 10 of 10 replicates from the 100 EB/ml level, 0 of 10 from the 10 EB/ml level and 0 of 10 at the 1 EB/ml level. Upon dilution of the processed material from the 100 EB/ml samples, Combo 2 detected CT in all 10 of 10 replicates from the 1:10, 1:100 and 1:1000 dilutions. LCx detected CT in 8 of 10 replicates from the 1:10 dilution, 2 of 10 from the 1:100 dilution and 0 of 10 from the 1:1000 dilution. COBAS detected CT in 8 of 10 replicates from the 1:10 dilution, 1 of 10 from the 1:100 dilution and 0 of 10 from the 1:1000 dilution. ProbeTec detected CT in 3 of 10 replicates from the 1:10 dilution, 0 of 10 from the 1:100 dilution and 0 of 10 from the 1:1000 dilution.

Comparison of Amplified Test Systems

Test	100 EB/ml	10 EB/ml	1 EB/ml
Combo2	10+/10	10+/10	10+/10
COBAS	10+/10	9+/10*	2+/10
LCx	10+/10	5+/10***	0+/10**
ProbeTec	8+/10	0+/10	0+/10

*1 gray zone positive **1 equivocal ***2 equivocal

Comparison of 100 EB/ml Diluted 1:10, 1:100, and 1:1000

Test	1:10	1:100	1:1000
Combo2	10+/10	10+/10	10+/10
COBAS	8+/10*	1+/10*	0+/10
LCx	8+/10**	2+/10**	0+/10
ProbeTec	3+/10	0+/10	0+/10

*1 gray zone positive **1 equivocal

CONCLUSION

These data indicate that significant differences in analytical sensitivity may occur among the amplification technologies studied, with Combo 2 assay showing the greatest sensitivity. The increased sensitivity of the Combo 2 assay may be due to combination of the following factors:

- Ribosomal RNA is present in the EB at approximately 2000 rRNA/EB in contrast to the DNA sequences targeted by the other assays that are typically present in several copies per EB. The increased rRNA target would initiate amplification in the Combo 2 assay even at high dilutions.
- All of the assays except Combo 2 require prerequisite centrifugation and decanting of the urine matrix prior to release of nucleic acids from CT EB. The centrifugation and decanting steps may lead to the loss of CT EB during specimen preparation, thus reducing or eliminating CT EB and the DNA targets.

These data suggest that the analytical sensitivity of the Combo 2 assay is at least one and possibly two logs higher than the other assays. This supports previous studies with clinical patient samples that have reported higher clinical sensitivity for the Combo 2 assay compared to other NAATs. (5,6)

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