Comparison of the COBAS AMPLICOR MTB and BDProbeTec ET Assays for Detection of *Mycobacterium tuberculosis* in Respiratory Specimens

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The performances of the BDProbeTec ET (Becton Dickinson) and COBAS AMPLICOR MTB (Roche) were retrospectively evaluated for detecting *Mycobacterium tuberculosis* complex in various respiratory specimens. The BACTEC and MGIT liquid culture system (Becton Dickinson) was used as a reference method. A total of 824 respiratory specimens, comprised of sputa, bronchoalveolar lavage fluid, and bronchial and tracheal aspirates from 580 patients, were evaluated. Out of 824 clinical specimens, 109 specimens from 43 patients were culture positive for *M. tuberculosis*. Of these 109 specimens, 67 were smear positive, 85 were positive by the COBAS AMPLICOR MTB test, and 94 were positive by the BDProbeTec ET. Of the 715 culture-negative specimens, 17 were positive by the auramine staining, 11 were positive by the COBAS AMPLICOR MTB test, and 12 were positive by the BDProbeTec ET. After discrepancy analysis and review of the patients’ clinical data, 130 specimens from 50 patients were considered “true-positive” specimens. This resulted in the following sensitivities: microscopy, 61.5%; COBAS AMPLICOR MTB test, 78.0%; and BDProbeTec ET, 86.2%. The specificities of each system, based on the clinical diagnosis, were 99.7% for microscopy, 99.9% for the COBAS AMPLICOR MTB test, and 99.9% for the BDProbeTec ET. The data presented represent a considerable number of specimens evaluated with a considerable number of culture- and auramine-positive and culture-positive and auramine-negative results and therefore give a realistic view of how the data should be interpreted in a daily routine situation. Specifically, the data with regard to the culture-positive and auramine-negative specimens are useful, because in a routine situation, auramine-negative specimens are sometimes accepted, on clinical indications, to be analyzed by an amplification method.

In contrast to general expectations, the incidence of mycobacterial disease has significantly increased worldwide since 1990 (7). The technical developments in diagnosing infections caused by *Mycobacterium tuberculosis* have increased similarly, an important change being the replacement of traditional solid culture media by liquid media and the introduction of molecular amplification techniques, such as PCR, which has decreased the turnaround time even further (1, 22). Many commercial assays are now available; for instance, the COBAS AMPLICOR MTB system (Roche, Basel, Switzerland) uses PCR (4, 27), while the BDProbeTec ET system (14) from Becton Dickinson ( Sparks, Md.) uses the “strand displacement amplification” technique for detecting *M. tuberculosis* (3, 5, 6, 8, 11, 23, 24, 26).

Amplification techniques have a good sensitivity for smear-positive specimens; however, for smear-negative samples, the reported sensitivity varies considerably (16, 17, 18, 21).

The objective of the present study was to compare the sensitivities and specificities of the BDProbeTec ET and the COBAS AMPLICOR MTB properly by evaluating the assays with the same set of processed specimens. Secondly, as the smear-negative specimens influence the overall sensitivities of the amplification methods, the accuracy of the two methods was studied in a considerable number of culture-positive and smear-positive specimens as well as in culture-positive but smear-negative specimens.

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MATERIALS AND METHODS

Specimens. Eight hundred twenty-four respiratory specimens (569 sputum samples or bronchial and tracheal aspirates and 255 bronchoalveolar lavage specimens) were obtained from 580 patients clinically suspected of suffering from tuberculosis. All specimens were obtained from two hospitals (the Erasmus University Medical Center and the St. Franciscus hospital) located in Rotterdam, The Netherlands.

Smear examination. Fixed smears of the digested and decontaminated specimens were stained with auramine-rhodamine (Becton Dickinson, Sparks, Md.) and examined by standard procedures for detection of acid-fast bacilli.

Specimen processing. After digestion, decontamination, and concentration, the specimens were suspended in phosphate-buffered saline (PBS) and, subsequently, one half of the volume was used for culture and one half was stored at −70°C ready for extraction of the DNA. All specimens were analyzed for the growth of mycobacteria by inoculation of the concentrated material into the MGIT or BACTEC system. Inoculation of the MGIT and BACTEC was performed according to the manufacturer’s instructions (Becton Dickinson, Sparks, Md.). Standardization in both centers was obtained using identical protocols with regard to specimen processing, auramine staining, and identification of positive cultures. The only difference between the two centers arises in the volume applied to resuspend the pellet after the NALC treatment procedure. This part of the procedure was standardized between the two centers by taking care that the initial PBS pellet suspension volumes of the pellets were equalized; i.e., in the Erasmus MC, the pellet suspension was made with up to 2 ml PBS. Otherwise, results of the amplification methods of the two centers were not comparable. As
quality controls, positive and negative amplification controls were included in each COBAS AMPLICOR or BDProbeTec ET run. The COBAS AMPLICOR and BDProbeTec ET procedures were followed in accordance with the manufacturer’s instructions (11, 12, 15).

Discrepancy analysis. A positive culture on Lowenstein-Jensen slants and/or in BACTEC or MGIT liquid culture systems which was also confirmed by acid-fast bacilli and AccuProbe was regarded as a true-positive specimen.

Any positive result obtained using the COBAS AMPLICOR MTB or BDProbeTec ET system that was not confirmed by a positive culture (solid or liquid medium) was regarded as a false-positive amplification result.

Positive results obtained using either COBAS AMPLICOR MTB or BDProbeTec ET system that were also confirmed by positive culture (solid or liquid medium) were regarded as true-positive amplification results.

Any specimen found to be positive with the COBAS AMPLICOR MTB system as well as with the BDProbeTec ET system but negative with solid or liquid medium was regarded as a true-positive result for tuberculosis culture with solid or liquid medium.

Culture-negative specimens that were positive with either the COBAS AMPLICOR MTB or the BDProbeTec ET system were regarded as M. tuberculosis positive when patients were clinically suspected of suffering from pulmonary tuberculosis and responding to antimicrobial therapy. Patients were also considered positive if a tuberculosis culture-positive specimen had been obtained (solid or liquid medium) in the last 6 months prior to the study.

RESULTS

The results of this evaluation are shown in Table 1. One hundred nine out of 824 specimens were culture positive for M. tuberculosis. Of these 109 culture positives, the COBAS AMPLICOR MTB system detected 85, and another 11 specimens were positive by this method in the M. tuberculosis culture-negative group. These results indicate that the independent sensitivity and specificity for the COBAS AMPLICOR MTB assay were 78 and 98.5%, respectively. Similarly, the BDProbeTec ET system detected 94 out of the 109 culture-positive specimens, with 12 positives among the culture-negative specimens. The sensitivity and specificity scores of the BDProbeTec ET were therefore calculated to be 86.2% and 98.3%, respectively. Interestingly, 17 culture-negative specimens were positive by at least one of the amplification systems used and 6 out of 17 of these negatives were actually positive by both amplification reactions. The remaining 11 discrepant results were resolved by studying the medical history of the patients from whom the 11 discrepant results came. These patients either had been clinically suspected of suffering from pulmonary tuberculosis, had responded to antimicrobial therapy, or had been pretreated for M. tuberculosis infection during the last 6 months. Consequently, 9 out of 11 COBAS AMPLICOR MTB or BDProbeTec ET positives with negative culture results were regarded as true positives, giving rise to identical specificities for both assays, i.e., 99.9%.

Thus, there appeared to be only one false-positive result using each amplification system for the 824 specimens tested. The sensitivities for culture-positive, auramine-negative, and auramine-positive specimens differ. Among the auramine-positive specimens, BDProbeTec ET detected 67/67 (100%) and the COBAS AMPLICOR MTB detected 64/67 (95.5%). For the auramine-negative specimens, a small difference was observed, as it was found that the BDProbeTec ET system detected 27/42 (64.3%) and the COBAS AMPLICOR MTB detected 21/42 (50%) auramine-negative specimens.

DISCUSSION

The primary objective of this study was to compare two amplification techniques in their ability to detect M. tuberculosis in respiratory specimens. Culture of M. tuberculosis in a liquid medium and auramine staining were the reference methods (2).

At the Erasmus MC site, the COBAS AMPLICOR MTB technique is routinely applied together with the MGIT as a liquid culture technique. At the other collaborating center, the BDProbeTec ET technique and the BACTEC liquid culture technique are applied together in the routine setting. After specimens had been processed for culture and microscopy, subsamples of the specimens were exchanged between the participating centers to be analyzed with the respective amplification methods (2).

Although the specimen volume was corrected, different results were obtained using the two amplification systems, as the COBAS AMPLICOR MTB system detected 85/109 (78%) and BDProbeTec ET system detected 94/109 (86.2%) culture-positive specimens. The majority of the differences found between the two systems came from the auramine-negative specimens. In 42 auramine-negative but culture-positive specimens, BDProbeTec ET detected 27 (64.3%) and the COBAS AMPLICOR MTB detected 21 (50%) positive specimens. The limited value of amplification assays in detecting true positives in auramine-negative specimens has been reported earlier by Piersimoni et al. (19).

Our results were comparable with the results obtained by Ichayama et al. (9), who reported sensitivities of 89.5% and 94.7% for COBAS AMPLICOR MTB and BDProbeTec ET, respectively. Similar results were obtained by Kim et al. (12); however, interpretation of these data is difficult, as only a limited number (n = 26) of positive specimens were studied. Inuma (10) did not find significant differences between the two amplification methods, but this is due to the fact that the specimens evaluated were almost all auramine positive. From our data, it is shown that there is no difference between the two amplification methods for the auramine-positive specimens. The differences between the amplification methods are observed with the auramine-negative specimens.

The COBAS AMPLICOR MTB and BDProbeTec ET systems detected additional positive specimens, though after
studying the medical history of the patients involved, we found that most of these additionally positive specimens came from patients who were undergoing treatment or who had been previously treated for *M. tuberculosis* during the last 6 months. Hellyer et al., Levee et al., and Thomsen et al. (8, 13, 25) previously demonstrated that *M. tuberculosis* DNA could be detected by strand displacement amplification and PCR in frozen sputum samples more than 12 months after the initiation of treatment and more than 6 months after positive-to-negative culture conversion.

The differences found between the two amplification systems could be explained by differences in the extraction methods used or by a reduction in the amount of inhibitory factors. The difference in sensitivity cannot be explained by differences in the volume used in the two amplification reactions. In the COBAS AMPLICOR MTB system, 25 μl of the primary extract is actually used in the amplification reaction. In the BDProbeTec ET assay, 13.75 μl is used. However, when the input volume is reduced, the input of inhibitory substances is also reduced, and the difference in sensitivities is therefore probably due to a better extraction method in combination with a reduction in inhibition, leading to optimized amplification. This presumably explains the absence of inhibited samples for the BDProbeTec ET assay in our study. In the COBAS AMPLICOR MTB system, seven specimens (0.85%) were reported to be inhibited, which leads to inconclusive results. Inhibition rates for the COBAS AMPLICOR MTB varying from 0.3% to 3.9% for respiratory and nonrespiratory specimens have been reported by others (20, 21, 25). In contrast to these findings, Kim et al. (12) reported an inhibition rate of 0.7% for BDProbeTec ET and of 0.0% for COBAS AMPLICOR. As the inhibition rates vary considerably, we suggest that the differences found between the two methods are probably due to a better extraction protocol applied by the BDProbeTec ET technique. Auramine-negative specimens have a low number of bacteria, and with these specimens, differences between results of the two methods are observed. Probably, due to the mechanical disruption applied by the BDProbeTec ET technique, more specific DNA of the organisms is extracted and becomes available for amplification.

At present, liquid culture techniques still appear to be superior to the amplification techniques in the diagnosis of *M. tuberculosis* infection. The difference in sensitivities between culture and molecular (standard PCR) testing is most probably due to the differences in input volume; culture is better due to a larger inoculum size. In our study, the advantage of the molecular techniques is lost for the auramine-negative group, i.e., the specimens with a low number of acid-fast bacilli.

From our results, the COBAS AMPLICOR MTB and BDProbeTec ET techniques have an added value for specimens that are also found to be auramine positive. In this respect, the COBAS AMPLICOR MTB and BDProbeTec ET amplification systems easily discriminate between *M. tuberculosis* and non-*M. tuberculosis* isolates, giving relevant information to the clinician with respect to diagnosis and optimal treatment of a particular patient. Both COBAS AMPLICOR MTB and BDProbeTec ET systems have an acceptable sensitivity and an excellent specificity. A difference in sensitivities between the results obtained for the present study and the results presented in previous publications may be attributed to the use of different reference methods, the use of different patient specimens (i.e., tissue specimens), or the inclusion or exclusion of auramine-negative specimens.

In conclusion, the sensitivities of the BDProbeTec ET system and the COBAS AMPLICOR MTB appear adequate for the analysis of auramine-positive samples. For auramine-negative samples, however, the number of false-negative results is still too high. Our results confirm the prevailing opinion that amplification techniques should currently serve only as an adjunct to culture and certainly not as a replacement.

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**REFERENCES**


