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Evaluation of the COBAS AMPLICOR MTB System

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A panel of 1,012 respiratory sediments was retrospectively tested by PCR amplification to detect *Mycobacterium tuberculosis* with the COBAS AMPLICOR MTB system. The sensitivities and specificities of COBAS and fluorescence microscopy compared to culture were 92.6 versus 95.6% and 99.6 versus 95.3%, respectively. Inhibition occurred in 48 (4.7%) specimens.

Introduction of molecular biology techniques, such as PCR, to the clinical mycobacteriology laboratory has had a strong impact in terms of the timeliness and accuracy of diagnostic results. However, the promise of rapid and sensitive detection of *Mycobacterium tuberculosis* directly from clinical specimens has as yet remained unfulfilled, because the sensitivity of current amplification assays still is below that of culture (3, 6, 7). Thus, their application to the routine diagnosis of tuberculosis from clinical samples remains limited.

Some features considered prerequisites for screening (1), such as partial automation and internal amplification control, have been implemented in the COBAS AMPLICOR MTB system. In addition, the automated system appears to be more sensitive and specific than its manual version (5). By retrospective analysis of frozen respiratory specimens, the performance of the COBAS AMPLICOR MTB system was evaluated.

A panel of 1,012 respiratory specimens (sputum, $n = 755$; bronchial secretions and washings, $n = 257$) from 480 patients was selected (Table 1) among sediments from the clinical laboratory where microbiology results and clinical data were concordant. The specimens had been decontaminated by the sodium dodecyl sulfate-sodium hydroxide method, examined for the presence of acid-fast bacilli (AFB) by fluorescence microscopy, and cultured with Bactec 12B and Loewenstein-Jensen medium as previously described (1). After processing, the remnants of the sediments were stored frozen at −70°C.

For PCR amplification, samples were prepared according to the instructions of the manufacturer (4). In short, 100 μl of sediment was washed in 500 μl of Sputum Wash Solution. After lysis at 60°C for 45 min in 100 μl of Sputum Lysis Reagent, the samples were neutralized by addition of 100 μl of Sputum Neutralization Reagent. Fifty microliters each of prepared patient specimens and negative and positive controls was transferred to tubes containing the master mix and loaded into the COBAS AMPLICOR MTB apparatus for the automated amplification and detection process. Amplification results of the *M. tuberculosis*-specific probe (MTB) and of the internal control (MCC) were recorded in optical density (OD) units (mOD; cut-off, 350 mOD). Inhibited specimens (MTB and MCC, <350 mOD) were retested after dilution (1:5) of the samples with a mixture (1:1) of Respiratory Lysis Reagent and Neutralizing Reagent according to the instructions of the manufacturer. Data were analyzed with Mann-Whitney U tests and box plots by using the StatView 4.02 software package (Abacus Concepts, Berkeley, Calif.).

The panel of 1,012 patient samples contained 229 (23%) sediments that yielded *M. tuberculosis* by culture. Most (95.6%) of these were smear positive. Thus, the proportion of positive sediments both by microscopy and by culture was much higher than usual in our clinical laboratory (1) because we primarily wanted to assess the system's ability to avoid carryover contamination during the automated amplification and detection procedure. Our results revealed that COBAS AMPLICOR MTB was very specific (99.6%) compared to culture results as the reference and that carryover apparently did not occur. The definitive assessment of specificity, however, would require the testing of a larger number of nontuberculous mycobacteria (NTM), including *Mycobacterium celatum*.

The sensitivity of COBAS AMPLICOR MTB in our panel was lower (91.3%) than that of fluorescence microscopy (95.6%) with culture results as the reference. Statistical analysis revealed that the cutoff value of 350 mOD discriminated most culture-negative samples and those containing NTM from those samples with a moderate-to-high *M. tuberculosis* content (AFB 2+ to AFB 4+; Fig. 1). In contrast, OD values of sediments containing fewer *M. tuberculosis* organisms (AFB negative to AFB 1+; Fig. 1) showed a broad overlap with the OD values of culture-negative specimens, explaining in part the surprisingly low sensitivity of COBAS AMPLICOR MTB. The use of a larger sample volume (e.g., 500 μl) might have a favorable influence on the amplicon detection characteristics and thus increase the sensitivity of the assay (2).

Frequent inhibition of the PCR amplification by samples of our panel could be another explanation for the low sensitivity. The internal amplification control allowed the simultaneous detection of sample inhibition. The systematic application of this option revealed inhibition of the PCR amplification in 48 (4.7%) of our sediments, including 4 smear-positive sediments containing *M. tuberculosis*. This rate was comparable to that reported for the manual version (6). In our view, this rate of inhibition justifies the systematic inclusion of the internal amplification control. On the other hand, the manufacturer's protocol proved to effectively overcome inhibition in 45 (94%) samples, thereby increasing the assay's sensitivity from 91.3% to 92.6%. Two smear-positive samples yielding *M. tuberculosis* by culture, however, remained inhibited despite pretreatment for inhibitors. Without the internal amplification control indicating sample inhibition, these specimens might have been misdiagnosed to contain NTM. This, in turn, might have prevented the prompt installation of respiratory isolation precautions and antituberculous drug therapy in the respective patients. In these cases, the internal amplification control thus contributed as well to the specificity of the assay.

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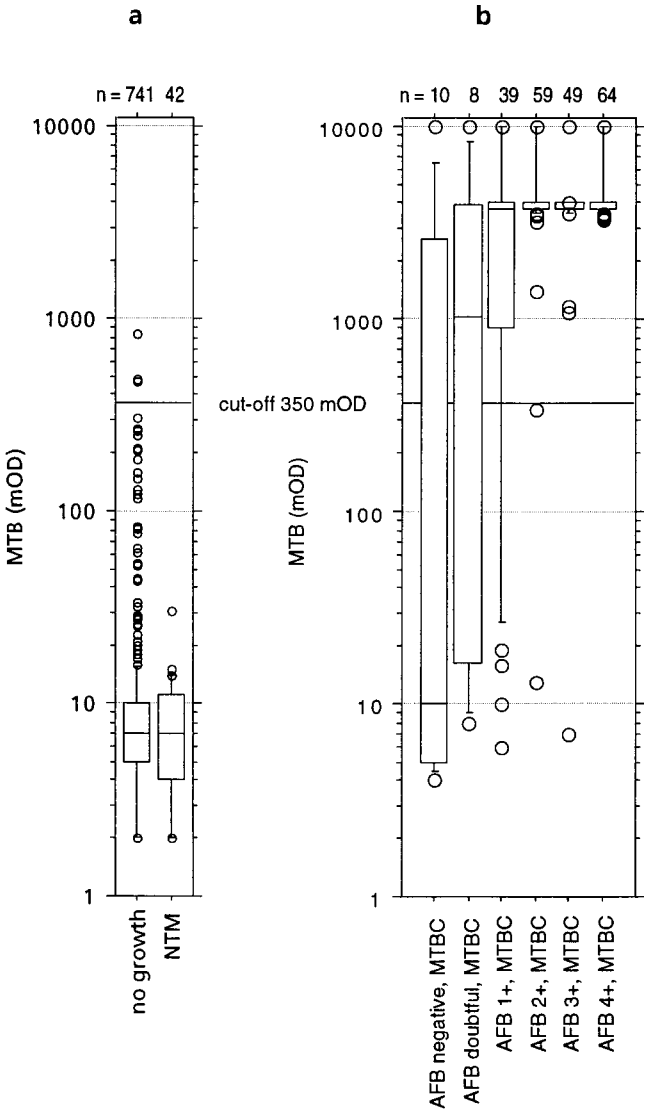

FIG. 1. ODs (mOD) of COBAS AMPLICOR MTB tests of 783 samples showing no growth of *M. tuberculosis* (MTBC) or growth of NTM (a) and 229 samples with growth of *M. tuberculosis* split by the results of fluorescence microscopy (b). OD values increased significantly (tied, $P < 0.05$ by Mann-Whitney U test) from AFB-negative MTBC to all other MTBC groups, except AFB-doubtful MTBC (tied, $P = 0.2$); from AFB-doubtful to AFB ≥2+ MTBC (tied, $P < 0.05$); and from AFB 1+ to AFB 2+ MTBC (tied, $P = 0.01$). The box plots show the median and 50% of the values within the boxes and 90% of the values within the bars.

Fifty microliters of each of the 20 specimens that were considered false negative by PCR was then tested for the presence of *M. tuberculosis*-specific RNA with a commercially available transcription-mediated amplification assay (Amplified Mycobacterium Tuberculosis Direct Test [AMTDT]; Gen-Probe, Inc., San Diego, Calif.) as previously described (1). AMTDT revealed positive results in 15 (75%) sediments and in 13 (93%) of the smear-positive sediments, including the five specimens that showed inhibition when tested by PCR. These results allowed us to rule out gross sample degradation due to improper storage conditions as the cause of false-negative PCR results. In addition, they suggested that AMTDT might be more sensitive and less prone to sample inhibition than COBAS AMPLICOR MTB.

TABLE 1. Smear and culture results of 1,012 sediments from respiratory specimens

No. of patients	*Mycobacterium* culture result	No. of smear results	
		Positive	Negative
75	*M. tuberculosis*	219	10
14	*M. avium* complex	23	2
1	*M. genavense*	0	1
1	*M. kansasii*	4	0
1	*M. szulgai*	4	0
1	*M. xenopi*	2	1
1	*M. gordonae*	1	0
4	Rapid growers	2	2
381	No growth	0	741

In conclusion, COBAS AMPLICOR MTB is a semiautomated PCR amplification system for the direct detection of *M. tuberculosis* in clinical specimens. While specific, its sensitivity was lower than that of fluorescence microscopy, particularly in specimens yielding only relatively few *M. tuberculosis* organisms. Systematic control for sample inhibition proved to be important and added to both the sensitivity and specificity of the assay.

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