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## Evaluation of Amplicor MTB Test as Adjunct to Smears and Culture for Direct Detection of *Mycobacterium tuberculosis* in the French Caribbean

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A total of 784 specimens collected from 370 individuals between January and August 1995 were analyzed by using the Amplicor *Mycobacterium tuberculosis* test (Roche Diagnostic Systems, Basel, Switzerland), a PCR-based test for the direct detection of organisms of the *M. tuberculosis* complex. The PCR results were compared with standard bacteriological data, including those obtained by acid-fast microscopy, culture, and biochemical identification as well as final clinical diagnosis for each patient. Several parallel controls were used: the kit DNA positive control,  $10^3$  CFU of *M. tuberculosis*, and three negative controls for each independent assay. No false-positive PCR results were obtained, and overall, *M. tuberculosis* was detected in 20 of 370 individuals screened. Five additional patients during the same time were found to be infected with mycobacteria other than tubercle bacilli; their specimens gave positive smear and/or culture test results, but Amplicor tests were always negative. The sensitivity, specificity, positive predictive value, and negative predictive value for the Amplicor MTB test compared with culture per specimen were 76.7, 97.7, 66.0, and 98.6%, respectively. For resolved cases, these values were, respectively, 69.4, 100, 100, and 96.8%; however, the sensitivity and negative predictive value increased to 90.9 and 99.2%, respectively, if PCR-negative nonrespiratory specimens (gastric washings) were not considered. When only specimens from proven tuberculosis patients were considered ( $n = 114$ ) and the sum of PCR-positive and/or culture-positive samples from proven tuberculosis patients was considered the total number of positive samples, PCR had a sensitivity of 83.3% compared with 71.6% for culture. Results per patient (about three samples each) yielded 100% sensitivity and 100% specificity. We conclude that the Amplicor MTB test is highly specific and rapid for routine use in a clinical laboratory. However, in order to obtain a high degree of sensitivity, it should be run as an adjunct to smears and culture with at least three samples for each patient, and a single-sample PCR-negative result must be considered carefully because of potential false-negatives.

The fatal association of a recent and an ancient scourge, AIDS and tuberculosis, is largely responsible for the recent interest in tuberculosis. Since the risk for a *Mycobacterium tuberculosis*- and human immunodeficiency virus-coinfected person to develop tuberculosis is about 100 times higher than for a person with normal host defenses, both the chemoprophylaxis and the treatment of tuberculosis have been the focus of recent investigations (5, 19).

With regard to geographic differences in epidemiologic patterns of human immunodeficiency virus transmission, the Caribbean islands fall into pattern II, which includes sub-Saharan Africa (18), with a recent increase in the incidence of tuberculosis since 1988 (4). Consequently, the Pasteur Institute started a specialized public health laboratory with reference activity in mycobacterial pathogens at Guadeloupe, French West Indies, in 1993 in order to circumvent any resurgence of tuberculosis and multidrug-resistant strains of *M. tuberculosis* in the Caribbean. As a part of this program, we undertook the present study to evaluate a PCR-based test for detection of *M. tuberculosis* in pathological specimens in the French Caribbean.

Recently, amplification of a specific DNA region of the 16S rRNA gene of *M. tuberculosis* followed by colorimetric detection has been commercialized as a kit (Roche Amplicor MTB) to allow a 1-day definitive identification of the tuberculosis pathogen (8, 24). The purpose of the present study was to

evaluate the Amplicor test with clinical specimens in parallel with standard staining, culture, and identification methods.

### MATERIALS AND METHODS

**Specimen collection and processing.** A total of 784 specimens from 370 individuals suspected of or known for having tuberculosis in Guadeloupe were studied. These specimens were sent to our laboratory for standard bacteriological confirmation between January and August 1995 and included sputum specimens, bronchial aspirates, and gastric washings. All specimens were stained with Ziehl-Neelsen stain, cultured in Löwenstein-Jensen medium after decontamination with sodium lauryl sulfate, and identified by standard procedures (9). The sodium lauryl sulfate decontamination method was shown to produce results comparable to those of the *N*-acetyl-L-cysteine-NaOH method with Amplicor in a recent study (6).

**The Amplicor MTB method.** The instructions provided by the manufacturer were followed in this investigation. Briefly, the Amplicor MTB test includes three major steps: a PCR target amplification (16, 20), hybridization of the amplified product to a specific nucleic acid probe, and colorimetric detection.

Genus-specific primers located in a highly conserved region of the 16S rRNA *Mycobacterium* gene (2, 22) are used to amplify a 584-bp sequence. Carryover contamination in the Amplicor method is prevented by incorporating dUTP in place of dTTP during amplification and utilization of uracil-*N*-glycosylase (AmpErase) to enzymatically cleave the uracil-containing DNA strands left from previous reactions prior to thermal cycling (15). This enzyme is subsequently inactivated at the temperatures used during thermal cycling.

Samples were prepared by adding 100  $\mu$ l of concentrated specimen to 500  $\mu$ l of sputum wash solution and were centrifuged at  $12,500 \times g$  for 10 min. The pellet was resuspended in 100  $\mu$ l of the lysis reagent, vortexed, heated for 45 min at 60°C, and neutralized by the addition of 100  $\mu$ l of neutralization reagent. For amplification, 50  $\mu$ l of neutralized specimen was added to an equal volume of master mix with AmpErase and amplified by using a TC-9600 thermal cycler (Perkin-Elmer, Norwalk, Conn.) as recommended by the manufacturer. After addition of 100  $\mu$ l of denaturation solution, the PCR tubes were incubated for 10 min at room temperature and 25  $\mu$ l of denatured amplicons was added to each well of the MTB probe-coated microwell plate containing 100  $\mu$ l of hybridization

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TABLE 1. Comparison of Amplicor MTB test and bacteriological results for 784 pathological samples from 370 individuals

Patient group	No. of specimens		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	PCR positive	PCR negative				
All except MOTT						
Smear positive	37	19	66.1	98.2	74.0	97.4
Smear negative	13	702				
Culture positive	33	10	76.7	97.7	66.0	98.6
Culture negative	17	711				
All <sup>a</sup>						
TB positive	50	22	69.4 (90.9 <sup>b</sup> )	100	100	96.8 (99.2 <sup>b</sup> )
TB negative	0	670				
Tuberculosis						
Smear positive	37	19	66.1 (88.1 <sup>c</sup> )	77.6	74.0	70.3 (90.0 <sup>c</sup> )
Smear negative	13	45				
Culture positive	33	10	76.7 (91.7 <sup>d</sup> )	76.1	66.0	84.4 (94.7 <sup>d</sup> )
Culture negative	17	54				
MOTT						
Smear positive	0	3	NA <sup>e</sup>	100	NA	NA
Smear negative	0	10				
Culture positive	0	8	NA	100	NA	NA
Culture negative	0	5				

<sup>a</sup> Tuberculosis (TB)-positive specimens came from confirmed tuberculosis patients who were smear and/or culture positive, whereas TB-negative specimens were from subjects not diagnosed as having tuberculosis. A total of 42 specimens from earlier tuberculous patients were excluded because they were simultaneously culture, smear, and PCR negative and came from patients most of whom had completed more than 1 month of treatment. These 42 specimens are included in the tuberculosis patient group.

<sup>b</sup> Excluding 17 PCR-negative gastric washing specimens.

<sup>c</sup> Excluding 14 PCR-negative gastric washing specimens.

<sup>d</sup> Excluding seven PCR-negative gastric washing specimens.

<sup>e</sup> NA, not applicable.

buffer. After 90 min of hybridization at 37°C, the microwells were washed five times with wash buffer, 100 µl of avidin-horseradish peroxidase conjugate was added, and the plates were reincubated for 15 min at 37°C and rewashed five times.

All washings were performed with an automated Multiwash-AR apparatus (Labsystems Ltd., Helsinki, Finland). For colorimetric detection, 100 µl of tetramethyl-benzidine-peroxidase solution was added, the wells were covered and incubated for 10 min at room temperature, and the reaction was stopped with 100 µl of stop reagent. The  $A_{450}$  was read with a Multiskan MS microplate reader (Labsystems Ltd.). An  $A_{450}$  of  $\geq 0.35$  was scored as a positive result, and an  $A_{450}$  of  $< 0.35$  was scored as a negative result.

In each independent assay, PCR test results were compared with results for two positive and three negative controls. The positive controls included the kit DNA control provided by Roche in one tube and a standardized suspension containing  $10^5$  CFU of *M. tuberculosis* in another reaction tube.

Recommendations by the manufacturer for prevention of contamination include distinct areas for reagent preparation (area 1), specimen preparation (area 2), and amplification and detection (area 3). In our case, these three areas were located in separate manipulation rooms. Furthermore, even specimen centrifugation was performed in a separate, aerosol-free room (distinct from both areas 1 and 2), which in our opinion is essential to avoid eventual false-positive results.

**Quantitative cultures and dilution endpoint.** The sensitivity of the Amplicor test in detecting the minimal number of organisms in a clinical specimen was also assayed. For this purpose, serial 10-fold dilutions in sterile distilled water of clinical isolates of *M. tuberculosis* grown on Löwenstein-Jensen were performed; 0.1-ml aliquots were plated onto complete Middlebrook 7H10 agar medium. The plates were incubated for 4 weeks at 37°C before CFU were counted. The sensitivity of the Amplicor PCR was determined by correlating the number of CFU of *M. tuberculosis* in serially diluted samples with the results of PCR performed on the same samples.

**Evaluation of effect of the Amplicor lysis procedure on bacterial viability.** In routine manipulations, there is always a possibility that some clinical specimens may contain very high numbers of mycobacteria, including organisms which may be particularly resistant to lytic procedures, e.g., *Mycobacterium avium*. Consequently, for safety reasons, we also evaluated the effect of the Amplicor lysis procedure on the viability of *M. tuberculosis* and *M. avium*. For this purpose, suspensions of clinical isolates of these two organisms were prepared as detailed above and the CFU in each sample were determined both before and after the lysis step.

## RESULTS

The PCR results for 784 specimens from 370 individuals were compared with standard bacteriological data obtained by acid-fast microscopy, culture, and biochemical identification as well as final clinical diagnosis for each patient and are summarized in Tables 1 and 2. During the course of this study, 20 of 370 individuals were positively diagnosed as suffering from *M. tuberculosis* disease (a total of 114 specimens). Five additional patients during the same time were found to be infected with mycobacteria other than tubercle bacilli (MOTT), and all 13 corresponding specimens gave positive smear and/or culture results, but the Amplicor tests were always negative. The low number of tuberculosis patients detected during the period of evaluation is in agreement with the low incidence of tuberculosis in Guadeloupe (11 cases per 100,000 people).

For specimens excluding MOTT (Table 1), the sensitivity,

TABLE 2. Comparison of Amplicor MTB test and culture results for the 370 individuals

Test and patient group	No. positive/ no. negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PCR		100	100	100	100
Tuberculous	20/0				
Nontuberculous	0/350				
Culture		80.0 (100 <sup>a</sup> )	100	100	98.6 (100 <sup>a</sup> )
Tuberculous	20/0				
Nontuberculous	5 <sup>b</sup> /345				

<sup>a</sup> Excluding MOTT.

<sup>b</sup> All cultures identified as MOTT.

TABLE 3. Minimum number of *M. tuberculosis* CFU detectable in 100- $\mu$ l samples with the Amplicor MTB kit

Viable count (CFU)	$A_{450}$ for strain:		
	1	2	3
$10^6$	3.15	3.14	3.10
$10^4$	3.40	3.33	3.35
$10^2$	1.97	3.25	3.22
2-10	2.87	3.09	3.17

specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Amplicor MTB test compared with culture were, respectively, 76.7, 97.7, 66.0, and 98.6%. When resolved results for all specimens, i.e., tuberculous or nontuberculous, were considered, the sensitivity, specificity, PPV, and NPV for the Amplicor MTB test were 69.4, 100, 100, and 96.8%, respectively (Table 1). However, since the Amplicor sample preparation kit was developed for respiratory tract specimens only and there exists only a claim by the manufacturer for these specimens, sensitivity and NPV were recalculated by excluding PCR-negative gastric washings, resulting in an increased sensitivity (90.9%) and NPV (99.2%). The complete absence of any false-positive PCR result during this study is noteworthy.

When all 114 specimens (sputum specimens, bronchial aspirates, and gastric washings) from proven tuberculosis patients were considered (Table 1), comparison of Amplicor test with smear and culture resulted, respectively, in sensitivities of 66.1 and 76.7%, specificities of 77.6 and 76.1%, PPVs of 74.0 and 66.0%, and NPVs of 70.3 and 84.4%. Exclusion of PCR-negative gastric washings for the reasons explained above resulted in increased sensitivities (88.1 and 91.7%) and NPVs (90.0 and 94.7%). It is noteworthy that PCR was able to detect 13 smear-negative and 17 culture-negative samples from patients for whom tuberculosis was bacteriologically confirmed in parallel or had been confirmed previously.

PCR and culture were also compared for their ability to detect *M. tuberculosis* in potentially positive samples. Considering that we had no false positives, the sum of PCR-positive and/or culture-positive samples was considered the total number of specimens containing tubercle bacilli, i.e., 33 + 17 + 10 = 60. In this case, PCR had a sensitivity of 83.3% compared with 71.6% for culture.

None of the 13 specimens from five patients from whom MOTT were isolated in culture (two *Mycobacterium fortuitum* isolates, one *Mycobacterium kansasii* isolate, one *Mycobacterium avium-M. intracellulare* isolate, and one *Mycobacterium chelonae* isolate) cross-reacted with the Amplicor test (Table 1). Consequently, the specificity of the Amplicor test in this case is 100%.

When results per patient were considered (Table 2), at least one PCR among two or three samples tested for each patient was positive, without any false positives. Thus, the results per patient yielded 100% sensitivity and specificity and 100% PPV and NPV. When results were considered for culture alone excluding MOTT, at least one positive culture per patient was obtained, yielding 100% sensitivity, specificity, PPV, and NPV.

The ability of the Amplicor test to detect the minimal number of organisms in a clinical specimen was assayed by correlating the number of CFU of *M. tuberculosis* organisms in serially diluted samples with PCR run in parallel. As shown in Table 3, an  $A_{450}$  of  $\geq 0.35$  (the cutoff value for a positive result) was obtained within a range of 2 to 10 CFU. These results are in agreement with those published recently involving clinical

specimens (25). Evaluation of the Amplicor lysis procedure for bacterial viability showed that the lysis procedure involved resulted in 100% killing of inocula of  $1.2 \times 10^6$  CFU of *M. tuberculosis* and  $1 \times 10^7$  CFU of *M. avium*.

## DISCUSSION

Efficient and timely treatment of tuberculosis is possible only if the delay in diagnosing the disease is reduced. Indeed, in four outbreaks of multidrug-resistant tuberculosis recorded by the Centers for Disease Control and Prevention in Florida and New York between 1988 and 1991, delayed laboratory identification of *M. tuberculosis* (mean time of 6 weeks from specimen collection) and recognition of drug resistance (mean time of 12 weeks, with up to 6 months in rare cases) led to considerable delays in initiating appropriate treatment (14). Thus, rapid detection and identification of *M. tuberculosis* would not only permit the start of appropriate treatment, but would also allow for isolation of patients in a timely manner to avoid nosocomial transmission of disease and drug resistance.

PCR can improve the rapid diagnosis of tuberculosis by allowing the direct detection of mycobacteria in clinical specimens. Several PCR-based tests and other amplification assays specific for mycobacteria have been reported (1, 3, 7, 10-13, 21, 23); however, a recent multicenter study involving PCR methodology using in-house primers reported an unexpectedly high variation in sensitivity, ultimately calling into question the reliability of such an approach for rapid detection of tuberculosis (17).

In the present investigation, we compared results of the recently commercialized Amplicor MTB test with standard bacteriological data obtained by acid-fast microscopy, culture, and biochemical identification as well as final clinical diagnosis of tuberculosis for each patient. No false-positive PCR results were obtained in this study. Even when the PCR test was positive and bacteriological data were negative, test material always came from proven tuberculosis patients. The test specificity per patient (two or three samples each) was 100% and remained unaltered even when smear- and/or culture-positive MOTT cases were considered.

Some of the smear- and culture-positive but PCR-negative results occurred with gastric washings in our study. Indeed, the Amplicor sample preparation kit was developed by the manufacturer uniquely for respiratory specimens and may not be fully adapted for gastric washings. Moreover, the eventual presence of PCR inhibitors in these samples cannot be excluded. We included gastric washings in our study because they are of great interest; they represented 85 of 114 specimens from confirmed tuberculosis patients. Among these 85 gastric washing specimens, 17 gave false-negative PCR results (10 smear positive, 3 culture positive, and 4 smear and culture positive), 32 were PCR positive, and 36 were probably true PCR negatives, as they were also smear and culture negative. Performance with nonrespiratory samples should be further evaluated, but our preliminary results suggest that gastric juices could potentially be assayed with the Amplicor MTB kit.

We conclude that the Amplicor test is at least as sensitive as culture for detection of *M. tuberculosis* on a per-specimen basis. In fact, PCR had a sensitivity of 83.3% compared with 71.6% for culture if the number of potentially positive samples is taken as the sum of PCR-positive and/or culture-positive samples. In our case, the Amplicor test was able to detect 13 cases of smear-negative and 17 cases of culture-negative tuberculosis. Under our conditions, a positive PCR test invariably detected a confirmed case of tuberculosis. On the other hand, PCR-negative data must be considered more carefully

because of false negatives. Moreover, the eventual presence of inhibitors of the PCR in some samples cannot be excluded. Analyzing more than one specimen per patient increases the chances of detecting *M. tuberculosis*, but the test still remains dependent on the number of organisms present in a sample.

We also found that the lysis method was effective even in the presence of a high number of bacilli and for specimens containing *M. avium*, making the method safe to use in a routine clinical microbiology laboratory. At the same time, the AmpliCor method was sensitive enough to release scant amounts of DNA from specimens containing as few as 2 to 10 bacilli which were then efficiently amplified and detected.

No clear link between a positive PCR test and active disease could be established in our study, as the test was found to be positive for some specimens from patients who had successfully completed up to 3 months of quadritherapy. These results suggest that PCR may also detect nonviable tubercle bacilli and are in agreement with recently published observations (25).

The complete absence of false positives in our study indicates that good laboratory practices as recommended by the manufacturer were fully implemented during the course of this investigation. Specimen centrifugation, moreover, was performed in a separate, aerosol-free room, which may further help to avoid eventual false positives. Last but not least, individual controls permitting the verification of the three major steps (sample lysis, amplification, and detection) in future development of the AmpliCor MTB kit should be included by the manufacturer. We also feel that greater automatization of the method would eventually avoid manual repeated steps, e.g., transfer of amplicons from the PCR tray to microwell detection plate and manipulation with irritant and harmful substances, such as the denaturation solution and the substrate 3,3',5,5'-tetramethylbenzidine, used for colorimetric detection. We also recommend that for better quality control, one should include at least one additional positive control containing 10<sup>3</sup> CFU of *M. tuberculosis*, which should be processed in parallel with the specimens to be tested. This would also permit an indirect control of the lysis step (in addition to amplification and detection steps as for the manufacturer's positive control containing DNA only).

In conclusion, with consideration of clinical and laboratory findings, AmpliCor is highly specific and rapid for routine use in a clinical laboratory. However, in order to obtain a high degree of sensitivity, it should be run as an adjunct to smears and culture with at least three samples for each patient. A PCR-negative result for a single specimen should be considered very carefully.

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#### REFERENCES

1. Abe, C., K. Hirano, M. Wada, Y. Kazumi, M. Takahashi, Y. Fukasawa, T. Yoshimura, C. Miyagi, and A. S. Goto. 1993. Detection of *Mycobacterium tuberculosis* in clinical specimens by polymerase chain reaction and Gen-Probe amplified Mycobacterium Tuberculosis Direct Test. *J. Clin. Microbiol.* **31**:3270–3274.
2. Böddinghaus, B., T. Rogall, T. Flohr, H. Blöcker, and E. C. Böttger. 1990. Detection and identification of mycobacteria by amplification of rRNA. *J. Clin. Microbiol.* **28**:1751–1759.
3. Brisson-Noel, A., C. Aznar, et al. 1991. Diagnosis of tuberculosis by DNA amplification in clinical practice evaluation. *Lancet* **338**:364–366.
4. Caribbean Epidemiology Center. 1993. Tuberculosis occurrence in CAREC member countries 1983–1992. CAREC Surveillance Rep. **19**:40–43.
5. Centers for Disease Control. 1992. Management of persons exposed to multidrug-resistant tuberculosis. *Morbidity and Mortality Weekly Report* **41**:61–71.
6. Codina, G., N. Martin-Casabona, T. Gonzalez, L. Arcalis, J. M. Manresa, and L. Sobejano. 1994. Evaluation of PCR in tuberculosis diagnosis, abstr. OP14, p. 33. In Proceedings of the European Society for Mycobacteriology, 15th Annual Meeting, Athens. Department of Microbiology, Athens Medical School, Athens, Greece.
7. Cousins, D. V., S. D. Wilton, B. R. Francis, and B. L. Gow. 1992. Use of polymerase chain reaction for rapid diagnosis of tuberculosis. *J. Clin. Microbiol.* **30**:255–258.
8. D'Amato, R. F., A. A. Wallman, L. H. Hochstein, P. M. Colaninno, M. Scardamaglia, E. Ardila, M. Ghouri, K. Kim, R. C. Patel, and A. Miller. 1995. Rapid diagnosis of pulmonary tuberculosis by using Roche AMPLICOR *Mycobacterium tuberculosis* PCR test. *J. Clin. Microbiol.* **33**:1832–1834.
9. David, H., V. Levy-Frébault, and M.-F. Thorel. 1989. Méthodes de laboratoire pour mycobactériologie clinique. Institut Pasteur, Paris.
10. Eisenach, K. D., M. D. Siford, M. D. Cave, J. H. Bates, and J. T. Crawford. 1991. Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. *Am. Rev. Respir. Dis.* **144**:1160–1163.
11. Hermans, P. W. M., A. J. Schuitema, D. V. Soolingen, C. P. H. J. Verstynen, E. M. Bik, J. E. R. Thole, A. H. J. Kolk, and J. D. A. van Embden. 1990. Specific detection of *Mycobacterium tuberculosis* complex strains by polymerase chain reaction. *J. Clin. Microbiol.* **28**:1204–1213.
12. Jonas, V., M. J. Alden, J. I. Curry, K. Kamisango, C. A. Knott, R. Landford, J. M. Wolfe, and D. F. Moore. 1993. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by amplification of rRNA. *J. Clin. Microbiol.* **31**:2410–2416.
13. Kennedy, N., S. H. Gillespie, A. O. S. Saruni, G. Kisyombe, R. M. Nermey, F. I. Ngowi, and S. Wilson. 1994. Polymerase chain reaction for assessing response in patients with pulmonary tuberculosis. *J. Infect. Dis.* **170**:713–716.
14. Kent, J. H. 1993. The epidemiology of multidrug-resistant tuberculosis in the United States. *Med. Clin. North Am.* **77**:1391–1409.
15. Longo, M. C., M. S. Beringer, and J. L. Hartley. 1990. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reaction. *Gene* **93**:125–128.
16. Mullis, K. B., and F. A. Faloona. 1987. Specific synthesis of DNA in vitro via a polymerase-catalysed chain reaction. *Methods Enzymol.* **155**:335–350.
17. Noordhoek, G. T., A. H. J. Kolk, G. Bjune, D. Catty, J. W. Dale, P. E. Fine, P. Godfrey-Fausset, S.-N. Cho, T. Shinnick, S. B. Svenson, S. Wilson, and J. D. A. van Embden. 1994. Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. *J. Clin. Microbiol.* **32**:277–284.
18. Piot, P., F. A. Plummer, F. S. Mhalu, J. L. Lamboray, J. Chin, and J. M. Mann. 1988. AIDS: an international perspective. *Science* **239**:573–579.
19. Rastogi, N., A. Kochi, B. Varedziz, K. Stybo, J. T. Crawford, W. R. Jarvis, J. E. McGowan, Jr., C. Perrone, H. L. David, Y. Zhang, D. L. Cohn, and M. D. Iseman. 1993. 9th Forum in Microbiology, "Emergence of multiple-drug-resistant tuberculosis: fundamental and applied research aspects, global issues and current strategies." *Res. Microbiol.* **144**:103–158.
20. Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim. 1985. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230**:1350–1354.
21. Sritharan, V., and R. H. Barker, Jr. 1991. A simple method for diagnosing *Mycobacterium tuberculosis* infection in clinical samples using PCR. *Mol. Cell. Probes* **5**:385–395.
22. Stahl, D. A., and J. W. Urbance. 1990. The division between fast- and slow-growing species corresponds to natural relationships among the mycobacteria. *J. Bacteriol.* **172**:116–124.
23. Thierry, D., C. Chureau, C. Aznar, and J.-L. Guesdon. 1992. The detection of *Mycobacterium tuberculosis* in uncultured clinical specimens using the polymerase chain reaction and a non-radioactive DNA probe. *Mol. Cell. Probes* **6**:181–191.
24. Vuorinen, P., A. Miettinen, R. Vuento, and O. Hällström. 1995. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by Gen-Probe amplified Mycobacterium Tuberculosis Direct Test and Roche AmpliCor Mycobacterium Tuberculosis Test. *J. Clin. Microbiol.* **33**:1856–1859.
25. Yajko, D. M., C. Wagner, V. J. Tevere, T. Kocagöz, W. K. Hadley, and H. F. Chambers. 1995. Quantitative culture of *Mycobacterium tuberculosis* from clinical sputum specimens and dilution endpoint by the AmpliCor PCR assay. *J. Clin. Microbiol.* **33**:1944–1947.