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Rapid and Specific Detection of *Mycobacterium tuberculosis* from Acid-Fast Bacillus Smear-Positive Respiratory Specimens and BacT/ALERT MP Culture Bottles by Using Fluorogenic Probes and Real-Time PCR

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A real-time PCR assay using the LightCycler (LC) instrument for the specific identification of *Mycobacterium tuberculosis complex* (MTB) was employed to detect organisms in 135 acid-fast bacillus (AFB) smear-positive respiratory specimens and in 232 BacT/ALERT MP (MP) culture bottles of respiratory specimens. The LC PCR assay was directed at the amplification of the internal transcribed spacer region of the *Mycobacterium* genome with real-time detection using fluorescence resonance energy transfer probes specific for MTB. The results from the respiratory specimens were compared to those from the Amplicor *M. tuberculosis* PCR test. Specimens from MP culture bottles were analyzed by Accuprobe and conventional identification methods. MTB was cultured from 105 (77.7%) respiratory AFB smear-positive specimens; 103 of these samples were positive by LC PCR and Amplicor PCR. Two samples negative in the LC assay contained rare numbers of organisms; both were positive in the Amplicor assay. Two separate samples negative by Amplicor PCR contained low and moderate numbers of AFB, respectively, and both of these were positive in the LC assay. There were 30 AFB smear-positive respiratory specimens that grew mycobacteria other than tuberculosis (MOTT), and all tested negative in both assays. Of the 231 MP culture bottles, 114 cultures were positive for MTB and all were positive by the LC assay. The remaining 117 culture bottles were negative in the LC assay and grew various MOTT. This real-time MTB assay is sensitive and specific; a result was available within 1 h of having a DNA sample available for testing.

Mycobacterium tuberculosis remains a serious public health issue due to its high risk of person-to-person transmission, morbidity, and mortality (15, 20). Currently, approximately 8 million new infections and 3 million deaths are attributed to tuberculosis (TB) each year (L. B. Reichman, Letter, Chest **112:**855, 1997). Progressive increases in TB infections are expected, and a worldwide annual incidence of 12 million cases by 2005 is predicted by the World Health Organization (20). The resurgence of TB in industrialized countries since the mid-1980s, primarily due to the increased incidence of immunocompromised patients with AIDS, and the emergence of multidrug-resistant strains of *M. tuberculosis* has accented the need for rapid diagnosis of this disease (15, 20). Rapid detection of active TB infection is critical for effective patient management and implementation of infection control measures.

Conventional detection of mycobacteria is based on a number of protocols, including microscopic examination of smears stained with the Ziehl-Neelsen stain or auramine fluorescent dye and selective culture techniques (8, 17, 26, 39). The key aspect of TB control is rapid diagnosis, which for many years has been based on the staining of smears for the presence of acid-fast bacilli (AFB). The AFB smear test lacks specificity, so there is a need for a laboratory test for specific detection of the *M. tuberculosis* complex (MTB) that can be performed within a short period of time.

Molecular methods, such as DNA probes and nucleic acid amplification tests, offer a rapid, specific, and sensitive approach to the detection of MTB from liquid cultures (29, 35) and for detection of TB directly from clinical specimens (1, 9, 11, 16, 22, 32, 37, 38). Nucleic acid amplification methods have been applied in the clinical laboratory with great success; however, these procedures are often labor intensive, and the FDA-approved nucleic acid amplification-based assays for MTB displayed high specificity but variable sensitivity (6, 7, 25). Multiple steps are required in the amplification and detection steps involving user manipulations at each point of the assay that have the potential for error and sample contamination.

Real-time PCR techniques, involving fluorescent dyes or fluorophores with a spectrofluorometric thermal cycler, have been used to develop a number of rapid and sensitive assays for identification of bacteria and viruses, including herpes simplex virus (12, 14), varicella-zoster virus (13), cytomegalovirus (33), *Legionella pneumophila* (2), enterohemorrhagic *Escherichia coli* (3), *Staphylococcus aureus* (28), *Mycobacterium bovis* (34) and also for genes conferring drug resistance in *Helicobacter pylori* (21), hepatitis B virus (5), *S. aureus* (28) and *M. tuberculosis* (36). Fluorogenic probes, including molecular beacons and paired hybridization probes, can be designed to recognize a specific sequence from a target gene and can enhance the specificity and sensitivity of the assay over those of conven-

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tional PCR techniques. Real-time fluorescence has been used to quantitate MTB DNA in sputum during treatment of TB patients using the TaqMan system (10). We recently reported a rapid and sensitive method for the identification of MTB by amplification of the internal transcribed spacer (ITS) and specific fluorogenic probes for MTB in the LightCycler (LC) system (19).

In this clinical laboratory-based study, specific identification of MTB was shown by using the LC PCR system (Roche Molecular Biochemicals) and real-time detection with specific fluorogenic probes. We were able to detect MTB DNA in processed AFB smear-positive respiratory specimens and MP culture bottles.

MATERIALS AND METHODS

Respiratory specimen processing and identification of mycobacteria. Respiratory specimens submitted for culture were liquified and decontaminated with *N*-acetyl cysteine–2.5% NaOH and concentrated by centrifugation (27). The sediment was used to inoculate a selective 7H11 agar plate and a supplemented MP culture bottle (Organon Teknika, Durham, N.C.) and to prepare two smears for staining. The respiratory, fixed smears were stained with an auramine fluorochrome stain (18). The number of fluorescent AFB was reported on the basis of the following criteria used in our laboratory (at a magnification of ×400): no AFB seen = negative; one to three per slide = 1+ (rare); one to nine per 10 fields = 2+ (few); one to nine per field = 3+ (moderate); and more than nine per field = 4+ (many) (24).

MP culture bottles were incubated at 35°C in 5% CO_2 and monitored for growth for 6 weeks by using the BacT/ALERT 3D instrument according to manufacturer's instructions. When growth was detected, a smear was prepared to confirm the presence of acid-fast organisms and the liquid medium was subcultured onto blood agar and a 7H11 plate. Isolates of mycobacteria growing on solid medium were identified by DNA probes (Accuprobe; Gen-Probe, Inc., San Diego, Calif.) for *M. tuberculosis*, *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium gordonae*, and *Mycobacterium kansasii* or by conventional biochemical tests performed according to standard protocols (18, 23).

DNA extraction for PCR protocols. Respiratory specimens that were AFB positive were prepared for PCR using the Roche sputum preparation kit (Roche Diagnostics, Indianapolis, Ind.). The remaining lysates were frozen at −20°C for use in the real-time PCR assay. Culture bottles that were AFB positive were prepared for PCR by using a Chelex 100 resin protocol. MP culture fluid (0.5 ml) was concentrated by centrifugation at 15,000 × *g* for 15 min in a 1.5-ml screw-cap microcentrifuge tube. The supernatant was removed, and the pellet was resuspended in 100 μl of TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]). This suspension was then transferred to a 1.5-ml tube containing 100 μl of a 40% solution of Chelex 100 resin (32) (Sigma Chemical, St. Louis, Mo.) in 10 mM Tris-HCl (pH 8.0) containing 0.2% laureth-12 (PPG industries, Gurnee, Ill.). The sample was resuspended by vortexing and incubated at 56°C for 30 min followed by incubation at 100°C for 10 min. The sample was vortexed again, allowed to cool, and centrifuged for 10 min to clarify the supernatant, which was transferred to another tube for storage prior to evaluation using real-time PCR.

AMPLICOR *M. tuberculosis* PCR test. AFB smear-positive respiratory specimens were processed for PCR directly from the decontaminated, concentrated sediment according to the package insert for the Amplicor *M. tuberculosis* test (Roche Diagnostics), as previously described (9). All manipulations of positive smear specimens were performed in a biological safety cabinet. PCR amplification and detection were performed according to manufacturer's guidelines. For this assay, 50 μl of the lysate was used for amplification in a total volume of 100 μl.

LightCycler real-time PCR for the ITS sequence. Oligonucleotides designated Sp1 (5′-ACCTCCTTTCTAAGGAGCACC-3′) and Sp2 (5′-GATGCTCG CAACCACTATCCA-3′) were used to amplify an approximately 220-bp fragment of the ITS sequence (EMBL accession number L15623) from *Mycobacterium* (30, 31). Amplified product was detected either by the use of SYBR Green I dye or by using specific fluorescent probes. The SYBR Green I dye allows detection of any double-stranded DNA generated during PCR; both specific and nonspecific products will generate a signal. In order to specifically identify MTB, paired fluorogenic hybridization probes were designed to recognize a region in the ITS fragment (19). The 5′ anchor probe was modified at the 3′ end with the donor fluorophore, 6-carboxy-fluoroscein, and designated 4602 (5′-GTGGGGC GTAGGCCGTGAGGGGTTC-FAM-3′). The 3′ detection probe was designated 4600 (5′-LC640-GTCTGTAGTGGGCGAGAGCCGGGTGC-*-3′) and was designed to hybridize 3 bp downstream of the anchor probe. The 3′ end of the probe was also phosphorylated (as indicated by the asterisk) to prevent amplification and extension with the 3′ SP2 primer. The hybridization probes were synthesized by Synthegen LLC (Houston, Texas).

For each sample, 2 μl of template DNA was incorporated into a 10-μl PCR containing the amplification oligonucleotides and MTB-ITS hybridization probes using the LightCycler DNA Master Hybridization kit (Roche Biochemicals). The optimized LC PCR protocol included an initial denaturation step at 95°C for 30 s and was followed by a touchdown PCR protocol using the following conditions: 95°C for 0 s, 61°C for 15 s, and 72°C for 30 s for five cycles; 95°C for 0 s, 60°C for 15 s, and 72°C for 30 s for 5 cycles; 95°C for 0 s, 59°C for 15 s, and 72°C for 30 s for five cycles; and 95°C for 0 s, 59°C for 5 s, and 72°C for 30 s for 35 cycles. The total time of amplification, detection, and analysis using this optimized protocol is approximately 40 to 45 min for 32 samples. Fluorescence measurements are made in every cycle. The threshold cycle (Ct) value is the cycle at which there is a significant increase in fluorescence, and this value is associated with an exponential growth of PCR product during the log-linear phase. Positive and negative controls were used that had been prepared by cloning the amplified ITS region into a pGEM vector from *M. tuberculosis* (pGEM MTB) and *M. kansasii* (pGEM MK), respectively (19). The total time for amplification, detection, and analysis is approximately 40 min for up to 32 samples per run.

TABLE 1. Detection of *M. tuberculosis* DNA from acid-fast smear positive respiratory samples by real-time PCR and Amplicor PCR

Real-time PCR/ Amplicor PCR	No. of samples with acid-fast smear result[a]				
	1+	2+	3+	4+	Total
Positive/positive	29	30	19	23	101
Negative/positive	2	0	0	0	2
Positive/negative	1	0	1	0	2

[a] Fluorescence microscopy (magnification, ×400). 1+, one to three per slide; 2+, one to nine per 10 fields; 3+, one to nine per field; 4+, more than nine per field.

RESULTS

Real-time MTB detection from concentrated respiratory specimens. Residual DNA lysates prepared for Amplicor MTB PCR from respiratory specimens that were AFB smear positive were tested in the LC PCR assay using 2 μl of the lysate (Table 1). We tested a total of 135 smear-positive specimens. There were 105 AFB smear-positive specimens tested that grew MTB. Of this group, 103 specimens were positive by LC PCR and Amplicor PCR. The two samples that were negative by LC PCR both contained low numbers of organisms by acid-fast smear and were negative on repeat testing. The two samples that were negative in the Amplicor PCR assay contained rare and moderate numbers of organisms by acid-fast smear, and both were positive in the LC assay. These samples were not tested further in the Amplicor PCR test, since both patients had other specimens that were positive in both PCR assays. There were 30 AFB smear-positive specimens tested that grew mycobacteria other than MTB, and all were negative in the LC PCR and Amplicor PCR assays. The sensitivity and specificity of the LC PCR assay for the AFB smear-positive specimens was 98.1% (103 of 105) and 100% (30 of 30), respectively.

The Ct, which is a measure of the cycle of detection of product, correlated with the acid-fast smear result. The average Ct value for 4+, 3+, 2+, and 1+ samples was 28.4, 30.4, 32.4, and 36.0 cycles, respectively.

TABLE 2. Detection of *M. tuberculosis* DNA from BacT/ALERT MP (MP) culture bottles by real-time PCR versus organism cultured from bottle

Organism cultured[a]	Result with real-time PCR		No. of patients
	No. positive	No. negative	
M. tuberculosis	114	0	41
MAI	0	67	43
M. kansasii	0	18	8
M. fortuitum	0	12	9
M. gordonae	0	6	5
M. chelonae	0	5	4
M. asiaticum	0	2	2
M. nonchromogenicum	0	2	2
M. simiae	0	1	1
M. kansasii/MAI	0	2	1
MOTT	0	2	2

[a] All are of the genus *Mycobacterium.* MOTT, mycobacteria other than tuberculosis.

Specific detection of MTB from MP culture specimens. We tested 231 MP cultures from respiratory specimens obtained from 118 patients. Of these, 114 were positive by LC PCR and all were culture positive for *M. tuberculosis* (Table 2). These samples were obtained from 41 patients. The remaining 117 specimens were negative in the LC assay. To examine for possible sample inhibition of amplification, all negatives samples were amplified using SYBR Green I, a fluorochrome that binds nonspecifically to double-stranded DNA. A melt curve analysis performed on the LC confirmed the presence of ITS fragment amplification in all specimens. All of these specimens were culture positive for various *Mycobacteria* species. At least eight different species were identified from these specimens. As expected for our institution, *M. avium* or *M. intracellulare (*MAI) and *M. kansasii* accounted for the majority of these isolates. The sensitivity and specificity of the LC PCR assay for the MP culture bottles specimens was 100% (114 of 114) and 100% (117 of 117), respectively.

The pGEM MTB plasmid was positive in all assay runs, and the pGEM MK plasmid and water negative controls were negative throughout these experiments. The average Ct value for the pGEM MTB plasmid was 23.2 (range of 18.7 to 25.4) over a 10-week period.

DISCUSSION

Due to the slow growth of *M. tuberculosis*, rapid identification methods using molecular techniques have been developed and utilized in the clinical laboratory. However, these methods require many manipulations and take several hours to complete. The LC instrument is a commercially available system designed to decrease the time of the PCR by monitoring the amplification of the target sequences in real time by fluorescent probes. This technology is a significant breakthrough in PCR amplification and amplicon detection compared to conventional detection methods, and the benefits for clinical assays have been reported (12, 13, 14, 33, 40).

Previously we utilized the LC and ITS oligonucleotide primers and probes on a collection of 20 different ATCC strains to determine the sensitivity and specificity of the assay (19). These strains represented the most commonly isolated species that are recovered in the clinical mycobacteriology laboratory. When the SYBR Green I dye was used as the fluorophore, we were unable to specifically identify MTB from among other *Mycobacterium* species. Several of the mycobacteria species produced amplified ITS products that had very unique melting points; however, most melting point profiles were too similar to reliably distinguish these species. The specific fluorescence resonance energy transfer probes we designed were shown to detect the MTB ITS sequence, while all other mycobacteria species tested negative. A plasmid containing the ITS sequence was prepared for MTB (pGEM MTB) and *M. kansasii* (pGEM MK). When the assay was performed using the pGEM MTB plasmid as the DNA template, we were consistently able to detect less than 200 fg per PCR. The pGEM MK plasmid, which was tested at a concentration of 2,000 pg per PCR, was always negative.

In this study, the LC real-time assay was evaluated for the specific identification of MTB from AFB smear-positive clinical specimens and from MP culture bottles. The LC assay was positive for 103 of 105 (98.1%) specimens that were culture positive for MTB. The two negative MTB samples both contained rare numbers of organisms by acid-fast smear and were submitted from two patients. For one of the patients, this specimen represented the only AFB smear-positive sample from several specimens that were submitted for this patient. All of the patient's cultures grew MTB. The other patient had two additional respiratory samples that were AFB smear positive; both of these samples were positive by the LC assay. Therefore, according to our clinical pathway for the diagnosis of TB in the hospital, this patient would have been correctly identified as positive for TB. We were impressed with the ability of the LC assay to detect positive patients because the sample size in this assay was much smaller than that in the Amplicor PCR assay. The latter test uses 50 μl of the lysed sediment, while the LC assay used only 2 μl of sample. Also, we did see a clustering of the Ct values that correlated with the acid-fast smear result. The greater the number of organisms present on the smear, the lower the Ct value for that specimen was. We have not evaluated this assay using AFB smear-negative samples. It is our feeling that these samples would require purification and concentration of the crude lysate using a silica membrane such as the QIAamp DNA mini kit (Qiagen, Valencia, Calif.). This method has been used to remove inhibitors in clinical specimens extracted by using the Amplicor protocol (4). In fact, we tested one acid-fast culture from a stool specimen and it was negative, possibly from inhibition. This specimen was from a patient who had respiratory samples that were positive in the LC assay.

The LC protocol proved very useful for the quick identification of MTB from MP culture bottles. Of the 231 liquid cultures that were tested in this study, 114 samples grew MTB and all were positive in the LC assay. The remaining 117 specimens were negative in the LC assay. The organisms isolated from these bottles included those that are commonly found in the clinical laboratory. MAI and *M. kansasii* accounted for 74% of these species.

In the LC assay, detection of amplified nucleic acid products is accomplished in a closed system; the capillary reaction vessels are never opened after the cycling process has started.

Therefore, there is no opportunity for carryover contamination to occur postamplification. There are steps in the extraction and processing procedures that may be susceptible to cross-contamination of target nucleic acid between specimens. To address this potential problem, specimen extraction would be performed in a biologic safety cabinet, and the sample loading should be done in a separate PCR workstation that is decontaminated with UV light after each use.

This LC assay proved to be very quick and potentially labor-saving in the laboratory. The time required for DNA extraction was 1.5 h, followed by 10 min for pipetting and 30 min for cycling and detection of amplified product. No other manual manipulations are necessary after the capillary reaction vessel was placed in the LC instrument. In contrast, the Amplicor *M. tuberculosis* PCR test took more than 5 h and required additional manual manipulation throughout the procedure. One hour was required for DNA extraction, 20 min was required for reagent preparation and pipetting of amplification mix, 1.8 h was required for amplification, and 2 h was required for detection by colorimetric microwell plate probe hybridization. Our goal is to implement this LC PCR method into our diagnostic laboratory for routine detection of MTB from AFB smear-positive specimens and MP cultures. Also, we would like to validate a protocol that we could use on AFB smear-negative specimens. Potentially, the combination of real-time PCR with sequence-specific fluorogenic probes can be optimized to detect mycobacterial DNA or RNA from sputum, bronchoalveolar lavage, blood, cerebrospinal fluid, pleural fluid, or tissue samples.

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REFERENCES

1. **Badak, F. Z., S. Goksel, R. Sertoz, B. Nafile, S. Ermertcan, C. Cavusoglu, and A. Bilgic.** 1999. Use of nucleic acid probes for identification of *Mycobacterium tuberculosis* directly from MB/BacT bottles. J. Clin. Microbiol. **37:**1602–1605.
2. **Ballard, A. L., N. K. Fry, L. Chan, S. B. Surman, J. V. Lee, T. G. Harrison, and K. J. Towner.** 2000. Detection of *Legionella pneumophila* using a real-time PCR hybridization assay. J. Clin. Microbiol. **38:**4215–4218.
3. **Bellin, T., M. Pulz, A. Matussek, H. G. Hempen, and F. Gunzer.** 2001. Rapid detection of enterohemorrhagic *Escherichia coli* by real-time PCR with fluorescent hybridization probes. J. Clin. Microbiol. **39:**370–374.
4. **Boddinghaus, B., T. A. Wichelhaus, V. Brade, and T. Bittner.** 2001. Removal of PCR inhibitors by silica membranes: evaluating the Amplicor *Mycobacterium tuberculosis* kit. J. Clin. Microbiol. **39:**3750–3752.
5. **Cane, P. A., P. Cook, D. Ratcliffe, D. Mutimer, and D. Pillay.** 1999. Use of real-time PCR and fluorimetry to detect lamivudine resistance-associated mutations in hepatitis B virus. Antimicrob. Agents Chemother. **43:**1600–1608.
6. **Centers for Disease Control and Prevention.** 2000. Update: nucleic acid amplification tests for tuberculosis. Morb. Mortal. Wkly. Rep. **49:**593–594.
7. **Centers for Disease Control and Prevention.** 1996. Nucleic acid amplification tests for tuberculosis. Morb. Mortal. Wkly. Rep. **45:**950–952.
8. **Chien, H. P., M. C. Yu, M. H. Wu, T. P. Lin, and K. T. Luh.** 2000. Comparison of the BACTEC MGIT 960 with Lowenstein-Jensen medium for recovery of mycobacteria form clinical specimens. Int. J. Tuberc. Lung Dis. **4:**866–870.
9. **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.
10. **Desjardin, L. E., Y. Chen, M. D. Perkins, L. Teixeira, M. D. Cave, and K. D. Eisenach.** 1998. Comparison of the ABI 7700 system (Taqman) and competitive PCR for quantification of IS*6110* DNA in sputum during treatment of tuberculosis. J. Clin. Microbiol. **36:**1964–1968.
11. **Ellner, P. D., T. E. Kiehn, R. Cammarata, and M. Hosmer.** 1988. Rapid detection and identification of pathogenic mycobacteria by combining radiometric and nucleic acid probe methods. J. Clin. Microbiol. **26:**1349–1352.
12. **Espy, M. J., T. K. Ross, R. Teo, K. A. Svien, A. D. Wold, J. R. Uhl, and T. F. Smith.** 2000. Evaluation of LightCycler PCR for implementation of laboratory diagnosis of herpes simplex virus infections. J. Clin. Microbiol. **38:**3116–3118.
13. **Espy, M. J., T. K. Ross, R. Teo, K. A. Svien, A. D. Wold, J. R. Uhl, and T. F. Smith.** 2000. Diagnosis of varicella-zoster virus infections in the clinical laboratory by LightCycler PCR. J. Clin. Microbiol. **38:**3187–3189.
14. **Espy, M. J., J. R. Uhl, P. S. Mitchell, J. N. Thorvilson, K. A. Svien, A. D. Wold, and T. F. Smith.** 2000. Diagnosis of herpes simplex virus infections in the clinical laboratory by LightCycler PCR. J. Clin. Microbiol. **38:**795–799.
15. **Falkinham, J. O.** 1996. Epidemiology of infection by nontuberculous mycobacteria. Clin. Microbiol. Rev. **9:**177–215.
16. **Gamboa, F., G. Fernandez, E. Padilla, J. M. Manterola, J. Lonca, P. J. Cardona, L. Matas, and V. Ausina.** 1998. Comparative evaluation of initial and new versions of the Gen-Probe Amplified *Mycobacteroim tuberculosis* Direct Test for direct detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens. J. Clin. Microbiol. **36:**684–689.
17. **Harris, G., A. Rayner, J. Blair, and B. Watt.** 2000. Comparison of three isolation systems for the culture of mycobacteria from respiratory and non-respiratory samples. J. Clin. Pathol. **53:**615–618.
18. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology: a guide for the level III laboratory. Centers for Disease Control, U.S. Department of Health and Human Services, Atlanta, Ga.
19. **Kraus, G., T. Cleary, N. Miller, R. Seivright, A. K. Young, G. Spruill, and H. J. Hnatyszyn.** 2001. Rapid and specific detection of *Mycobacterium tuberculosis* using fluorogenic probes and real-time PCR. Mol. Cell. Probes **15:**375–383.
20. **Martin, G., and A. Lazarus.** 2000. Epidemiology and diagnosis of tuberculosis. Recognition of at-risk patients is key to prompt detection. Postgrad. Med. **108:**42–44, 47–50, 53–54.
21. **Matsumura, M., Y. Hikiba, K. Ogura, G. Togo, I. Tsukuda, K. Ushikawa, Y. Shiratori, and M. Omata.** 2001. Rapid detection of mutations in the 23S rRNA gene of *Helicobacter pylori* that confers resistance to clarithromycin treatment to the bacterium. J. Clin. Microbiol. **39:**691–695.
22. **Metchock, B., and L. Diem.** 1995. Algorithm for use of nucleic acid probes for identifying *Mycobacterium tuberculosis* from BACTEC 12B bottles. J. Clin. Microbiol. **33:**1934–1937.
23. **Metchock, B. G., F. Nolte, and R. J. Wallace.** 1999. Mycobacterium, p. 399–437. *In* P. R. Murray, E. J. Baron, M. A. Phaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.
24. **Miller, N., S. G. Hernandez, and T. J. Cleary.** 1994. Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test and PCR for direct detection of *Mycobacterium tuberculosis* in clinical Specimens. J. Clin. Microbiol. **32:**393–397.
25. **Noordhoek, G. T., H. A. Kolk, G. Bjune, G., D. Catty, J. W. Dale, P. E. Fine, P. Godfrey-Faussett, S. N. Cho, T. Shinnick, and S. B. Svenson.** 1994. Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. J. Clin. Microbiol. **32:**277–284.
26. **Pfyffer, G. E., H. M. Welscher, P. Kissling, C. Cieslak, M. J. Casal, J. Gutierrez, and S. Rusch-Gerdes.** 1997. Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. J. Clin. Microbiol. **35:**364–368.
27. **Ratnam, S., F. A. Stead, and M. Howes.** 1987. Simplified acetylcysteine-alkali digestion-decontamination procedure for isolation of mycobacteria from clinical specimens. J. Clin. Microbiol. **25:**1428–1432.
28. **Reischl, U., H. J. Linde, M. Metz, B. Leppmeier, and N. Lehn.** 2000. Rapid identification of methicillin-resistant *Staphylococcus aureus* and simultaneous species confirmation using real-time fluorescence PCR. J. Clin. Microbiol. **38:**2429–2433.
29. **Reisner, B. S., A. M. Gatson, and G. L. Woods.** 1994. Use of Gen-Probe Accuprobe to identify *Mycobacterium avium* complex, *Mycobacterium tuberculosis* complex, *Mycobacterium kansasii*, and *Mycobacterium gordonae* directly from BACTEC TB broth cultures. J. Clin. Microbiol. **32:**2995–2998.
30. **Roth, A., M. Fischer, M. E. Hamid, S. Michalke, W. Ludwig, and H. Mauch.** 1998. Differentiation of phylogenetically related slowly growing mycobacteria based on 16S-23S rRNA gene internal transcribed spacer sequences. J. Clin. Microbiol. **36:**139–147.
31. **Roth, A., U. Reischl, A. Streubel, L. Naumann, R. M. Kroppenstedt, M. Habicht, M. Fischer, and H. Mauch.** 2000. Novel diagnostic algorithm for identification of mycobacteria using genus-specific amplification of the 16S-23S rRNA gene spacer and restriction endonucleases. J. Clin. Microbiol. **38:**1094–1104.
32. **Scarparo, C., P. Piccoli, A. Rigon. G. Ruggiero, D. Nista, and C. Piersimoni.** 2001. Direct identification of mycobacteria from MB/BacT Alert 3D bottles: comparative evaluation of two commercial probe assays. J. Clin. Microbiol. **39:**3222–3227.
33. **Schaade, L., P. Koekelkorn, K. Ritter, and M. Kleines.** 2000. Detection of

cytomegalovirus DNA in human specimens by LightCycler PCR. J. Clin. Microbiol. **38:**4006–4009.
34. **Taylor, M. J., M. S. Hughes, R. A. Skuce, and S. D. Neill.** 2001. Detection of *Mycobacterium bovis* in bovine clinical specimens using real-time fluorescence and fluorescence resonance energy transfer probe rapid-cycle PCR. J. Clin. Microbiol. **39:**1272–1278.
35. **Telenti, M., J. F. de Quiros, M. Alvarez, M. J. Santos Rionda, and M. C. Mendoza.** 1994. The diagnostic usefulness of a DNA probe for *Mycobacterium tuberculosis* complex (Gen-Probe) in Bactec cultures versus other diagnostic methods. Infection **22:**18–23.
36. **Torres, M. J., A. Criado, J. C. Palomares, and J. Aznar.** 2000. Use of real-time PCR and fluorimetry for rapid detection of rifampin and isoniazid resistance-associated mutations in *Mycobacterium tuberculosis*. J. Clin. Microbiol. **38:**3194–3199.
37. **Tortoli, E., F. Lavinia, and M. T. Simonetti.** 1997. Evaluation of a commercial ligase chain reaction kit (Abbott LCx) for direct detection of *Mycobacterium tuberculosis* in pulmonary and extrapulmonary specimens. J. Clin. Microbiol. **35:**2424–2426.
38. **Wang, S. X., and L. Tay.** 1999. Evaluation of three nucleic acid amplification methods for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. J. Clin. Microbiol. **37:**1932–1934.
39. **Williams-Bouyer, N., R. Yorke, H. I. Lee, and G. L. Woods.** 2000. Comparison of the BACTEC MGIT 960 and ESP culture system II for growth and detection of mycobacteria. J. Clin. Microbiol. **38:**4167–4170.
40. **Wittmer, C. T., M. G. Herrmann, A. A. Moss, and R. P. Rasmussen.** 1997. Continuous fluorescence monitoring of rapid cycle DNA amplification. BioTechniques **22:**130–138.