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Genus Level Identification of Mycobacteria from Clinical Specimens by Using an Easy-To-Handle *Mycobacterium*-Specific PCR Assay

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An easy-to-handle *Mycobacterium*-specific PCR assay for detection of the presence of a wide range of mycobacterial species in clinical samples was evaluated. The performance of the genus probe was compared with the performance of probes specific for *Mycobacterium tuberculosis* and *Mycobacterium avium* and with that of standard culture. In addition, the utility of an internal control in monitoring amplification inhibitors was studied. Of 545 respiratory and 325 nonrespiratory specimens (a total of 870 specimens), 58 (6.7%) showed the presence of amplification inhibitors, as determined by a negative result for the internal control. Of these 58 specimens, 31 (53%) were stool specimens; other material, even citrate blood after lysis of erythrocytes, did not pose a problem with regard to inhibition of PCR amplification. Eighty-one of the remaining 812 specimens had a positive *Mycobacterium* culture result. Of these culture-positive specimens, 58 (71.6%) showed a positive result with the *Mycobacterium* genus-specific probe. Seventy-two samples had a positive result with the *Mycobacterium*-specific probe but a negative culture result. Of these 72 samples, 26 samples were regarded as true positive, either because the *M. tuberculosis*- or *M. avium*-specific probe was also positive at the same time or because other specimens from the same patient taken at the same time were culture positive. The sensitivity of the *Mycobacterium*-specific probe was 78.5% and the specificity was 93.5%. This study showed that pretesting of clinical specimens for mycobacteria to the genus level with a *Mycobacterium*-specific probe offers the routine clinical laboratory the possibility of detecting tuberculous and nontuberculous mycobacteria with one test. Furthermore, specimens testing positive with the genus-specific probe can be immediately identified with species-specific probes.

The need for rapid laboratory diagnosis of tuberculosis has led to the development of a number of amplification-based molecular diagnostic procedures for detecting and identifying *Mycobacterium tuberculosis*. Various easy-to-use kits for *M. tuberculosis* detection and identification based on nucleic acid amplification techniques are commercially available, such as the AMPLICOR MTB (PCR) kit of Roche (5, 30), the MTD (isothermal amplification of rRNA) kit of GenProbe (8, 22), and the newly introduced ligase chain reaction of Abbott Laboratories (available in Europe).

In addition to the resurgence of tuberculosis in developing and developed countries (1), there remains a high rate of recovery of mycobacteria other than *M. tuberculosis* (MOTT) from clinical specimens (23, 25, 34, 35). MOTT play a particularly important role in patients with AIDS (14), in whom *M. tuberculosis* is also often encountered as an infectious agent (15, 31). It is therefore of great importance for a microbiological laboratory which routinely diagnoses mycobacterial infections to detect *M. tuberculosis* and MOTT at a very early stage of infection.

Numerous amplification assays for the specific detection of different species of mycobacteria have been reported (4, 6, 7, 9, 11, 13, 26, 27, 29). However, use of a battery of species-specific assays is impractical, especially in a clinical laboratory setting. Single assays that can detect and identify multiple mycobacterial species have been reported (2, 10, 12, 20, 21, 32). Unfortunately, the species identification methods used in these assays are cumbersome and are not easily accommodated in the routine work of the clinical microbiology laboratory. The development of an assay with pan-genus primers for the amplification of DNA from most species of mycobacteria and species-specific probes for species identification was reported recently (33). The pan-genus primers are not absolutely specific for the genus *Mycobacterium*, and DNAs from some species of closely related genera such as *Corynebacterium* and *Nocardia* are amplified. We report here the development of a *Mycobacterium*-specific probe that can be used in conjunction with the pan-genus primers to detect the presence of a wide range of mycobacterial species.

The performance of the probe in detecting mycobacteria in clinical specimens was evaluated. The performance of the genus-specific probe was compared with the performance of probes specific for *M. tuberculosis* and *M. avium* and with that of standard culture. We also determined whether the specificity of the probe is acceptable for a routine laboratory and to what extent this test is able to detect MOTT in clinical specimens. To this end, respiratory and nonrespiratory specimens were tested at the same time after amplification with the *Mycobacterium*-, *M. tuberculosis*-, and *M. avium*-specific probes. Direct microscopic examination (except stools, blood, and

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urine) and culture (culture on solid media and radiometric culture) were performed for all specimens.

Another aspect of the current study examined the utility of an internal control (IC) in monitoring amplification inhibitors. The presence of inhibitors can compromise the performance of nucleic acid amplification assays, leading to false-negative results. Because the internal control can be amplified by the target-specific primers but is distinguishable from the target by a unique probe-binding site, it is useful for routinely monitoring for the presence of amplification inhibitors. The internal control is introduced into the amplification mixture and is coamplified with the specific target. In specimens negative for the target of interest, a positive IC signal indicates the absence of inhibitors and a true negative result. On the other hand, a negative IC signal indicates the presence of inhibitors and the possibility of a false-negative result. To ensure the validity of the results in the clinical evaluation described above, the IC was coamplified in all specimens.

MATERIALS AND METHODS

Specimens. Respiratory specimens (sputa and bronchial secretions obtained by bronchoscopy) and nonrespiratory samples comprising pleural exudates, lymph node, skin, or pleural biopsy specimens, and gastric aspirates, citrate blood, and stool specimens were included in this study. The specimens were kept at 2 to 8°C until they were ready for processing. Gastric aspirates were immediately neutralized with trisodium phosphate buffer (pH 12.0) after retrieval. Tissue specimens were sliced with a scalpel into small pieces and homogenized in a mortar under sterile conditions before processing. Citrate blood was diluted 1:1 with distilled water directly after arrival in the laboratory, shaken for 20 min at room temperature, and centrifuged at 3,500 × *g* for 25 min. The supernatant was discarded, and the procedure was repeated four times. Decontamination of all specimens except blood was performed by the NaOH–*N*-acetyl-L-cysteine (NALC) procedure (17). An equal volume of digestant (0.0306 M NALC [Sigma Chemical Co., St. Louis, Mo.], 0.05 M trisodium citrate, 1 M NaOH) was added to each specimen, after which the specimens were briefly vortexed and then shaken for 20 min. The specimens were then diluted with distilled water and centrifuged at 3,500 × *g* for 25 min. The supernatant was discarded and the sediment was resuspended in 2 ml of distilled water.

Two hundred microliters of each resuspended sediment was removed and was kept at 2 to 8°C for subsequent PCR testing, which was performed at least once a week. One hundred microliters was stored at −20°C. The remaining sediment was used for acid-fast staining and culture.

Microscopy. Smears were stained with auramine-rhodamine fluorochrome. Positive staining was confirmed by the Ziehl-Neelsen technique (17).

Culture. One milliliter (total) of the sediment was inoculated onto one slant each of Lowenstein-Jensen medium and Stonebrink medium (both media were produced in our laboratory), and a further 0.3 ml was inoculated into BACTEC vials supplemented with 0.1 ml of polymyxin B, nalidixic acid, trimethoprim, and azlocillin. Slants and vials were incubated at 36 ± 1°C for up to 8 weeks. For the identification of mycobacteria, the nitro-α-acetyl-amino-β-hydroxy-propiophenone test and other routine biochemical methods were used.

PCR. (i) Specimen preparation. After decontamination, all specimens except stool specimens were processed with reagents from the AMPLICOR MTB test (Roche Diagnostic Systems, Somerville, N.J.) following the manufacturer's instructions. Briefly, 500 μl of wash solution was added to 100 μl of each decontaminated specimen. In the case of the stool specimens, a 1:10 dilution of the original specimen was made with wash solution; otherwise, the procedure was identical to that for the other specimens. The mixture was vortexed and centrifuged at 12,500 × *g* for 10 min. The supernatant was aspirated and 100 μl of lysis reagent was added to the pellet. The samples were then vortexed and incubated at 60°C in a heat block for 45 min. After the incubation, the tubes were pulse centrifuged at 12,500 × *g* for 10 s, and 100 μl of neutralization reagent was added, followed by vortexing. Fifty microliters of the prepared specimens were amplified with 50 μl of a premade amplification mixture (33) containing 20 copies of the IC.

(ii) Amplification. Amplification reactions were carried out in a GeneAmp System 9600 thermal cycler (Perkin-Elmer, Norwalk, Conn.) as described previously (33). The reaction mixtures were incubated at 50°C for 2 min, followed by 2 cycles consisting of 20 s at 98°C, 20 s at 62°C, and 45 s at 72°C and 35 cycles consisting of 20 s at 94°C, 20 s at 62°C, and 45 s at 72°C. After the final cycle, the tubes were incubated for an additional 5 min at 72°C. The amplification products were denatured with 100 μl of denaturation solution, and the samples were stored overnight at 4°C.

(iii) Detection. Amplification products were hybridized to probes used to coat microwell plates as described previously (19). Briefly, 25 μl of the denatured, amplified specimen was transferred to the wells of microwell plates (one plate each was coated with the *Mycobacterium*-, *M. tuberculosis*-, or *M. avium*-specific probe or the IC probe) containing 100 μl of hybridization buffer. The plates were covered and incubated for 1.5 h at 37°C. At the end of the incubation period, the plates were washed five times. One hundred microliters of avidin-horseradish peroxidase conjugate was added, followed by incubation for 15 min at 37°C. The washing procedure was repeated and 100 μl of substrate was added, followed by incubation for 10 min at room temperature. The reaction was stopped with 4.9% H_2SO_4, and the optical density was measured at 450 nm in a microtiter plate reader. A cutoff value of 0.35 was used for all probes.

Analysis of data. The results obtained with the *Mycobacterium*-specific probe were compared with those obtained with the *M. tuberculosis*- and *M. avium*-specific probes and with the results of culture (on Lowenstein-Jensen, Stonebrink, and BACTEC media). True *Mycobacterium*-specific probe-positive specimens were defined as those which tested positive at the same time with the *Mycobacterium*-specific probe and with one other probe tested and/or by culture. Also counted as true positives were those specimens collected from any patient who, at the same time of specimen collection, gave another culture-positive specimen.

TABLE 1. Numbers and types of specimens that inhibit amplification

Specimen	No. of specimens that were inhibitory/total no. of specimens (% inhibitory specimens)
Respiratory	
Sputa	7/281 (2.5)
Bronchial secretion	5/264 (1.9)
Total respiratory	12/545 (2.2)
Nonrespiratory	
Stool	31/43 (72.1)
Biopsy	5/42 (11.9)
Citrate blood	4/49 (8.2)
Urine	2/88 (2.3)
Bone marrow	2/2 (100)
Gastric juice	1/33 (3.0)
Pleural effusion	1/40 (2.5)
Pus	0/3 (0)
Cerebrospinal fluid	0/25 (0)
Total nonrespiratory	46/325 (14.2)
Total for all specimens	58/870 (6.7)

RESULTS

Frequency of inhibitory specimens. A total of 545 respiratory and 325 nonrespiratory specimens (a total of 870 specimens) were included in this study. The bronchopulmonary samples consisted either of expectorated or induced sputa (n = 281) or bronchial secretions (n = 264) obtained by bronchoscopy. The nonrespiratory samples included urine (n = 88), citrate blood (n = 49), stool (n = 43), biopsy specimens of tissues (lymph node, skin, and pleura) (n = 42), pleural exudates (n = 40), aspirates of gastric juice (n = 33), cerebrospinal fluid (n = 25), pus (n = 3), and bone marrow (n = 2).

Fifty-eight (6.7%) of the specimens showed the presence of amplification inhibitors, as determined by negative IC signals (Table 1). Of these 58 specimens, 31 (53%) were stool specimens, a result which was not unexpected since stool specimens are known to be inhibitory to PCR amplification. Other material, even citrate blood after lysis of erythrocytes, did not seem to pose a problem with regard to inhibition of PCR amplification. Excluding stool specimens, only 3.3% of all specimens tested, or 2.2% of respiratory and 5.3% of nonrespiratory specimens, inhibited PCR amplification. For the purpose of this clinical study, only results for specimens showing no evidence of inhibition were included in the evaluation.

Performance of the *Mycobacterium*-specific probe. No specimens showing a positive result with the *M. tuberculosis*- or

TABLE 2. Different types of clinical material showing positive culture results

Mycobacterial species	Sample	No. of samples
M. tuberculosis	Sputum	35
	Bronchial secretion	16
	Biopsy	3
	Pleural effusion	3
	Urine	2
	Gastric juice	2
M. avium	Biopsy	8
	Sputa	2
	Stool	1
	Blood	1
Other mycobacteria[a]	Sputa	4
	Bronchial secretion	3
	Biopsy	1

[a] *M. xenopi*, (n = 5), *M. marinum* (n = 1), *M. gordonae* (n = 1), and a *Mycobacterium* sp. (n = 1).

M. avium-specific probe had a negative result with the *Mycobacterium*-specific probe. Eighty-one of the 812 noninhibitory specimens had a positive *Mycobacterium* culture result (Table 2). More specifically, 61 specimens grew *M. tuberculosis*, 12 grew *M. avium*, and 8 others grew mycobacteria (5 grew *M. xenopi*, 1 grew *M. marinum*, 1 grew *M. gordonae*, and one grew a *Mycobacterium* sp.). The different types of clinical material giving a positive culture result are listed in Table 2. Of the 81 culture-positive specimens, 58 (71.6%) had a positive *Mycobacterium*-specific probe result. Among the 61 *M. tuberculosis* culture-positive specimens, 43 were positive with the *Mycobacterium*-specific probe, in contrast to 37 which gave positive results with the *M. tuberculosis*-specific probe. Only 9 of 59 (15.3%) *M. tuberculosis* culture-positive specimens had a positive smear result (2 urine specimens were not examined by microscopy). All nine smear-positive specimens were positive with the *Mycobacterium*-specific probe and the *M. tuberculosis*-specific probe. Among the 12 samples culture positive for *M. avium*, 9 were positive with the *Mycobacterium*-specific probe and 7 were positive with the *M. avium*-specific probe. For the eight specimens from which other mycobacteria could be grown in culture, six (three that grew *M. xenopi*, one that grew *M. marinum*, one that grew *M. gordonae*, and one that grew *Mycobacterium* sp.) were positive with the *Mycobacterium*-specific probe (Table 3). The performance of the *Mycobacterium*-specific probe was similar with both respiratory and nonrespiratory specimens (data not shown). The results of culture and the results obtained with the genus- and species-specific probes for the 812 noninhibitory specimens are as follows: 81 specimens were positive and 731 specimens were negative by culture. With the *Mycobacterium*-, *M. tuberculosis*-, and *M. avium*-specific probes, 130, 50, and 8 specimens, respectively, were positive and 682, 762, and 804 specimens, respectively, were negative.

Seventy-two samples had positive *Mycobacterium*-specific probe results but negative culture results. Thirteen of these samples were also positive with the *M. tuberculosis*-specific probe and one was positive with the *M. avium*-specific probe. For 11 other patients, other specimens taken at the same time were culture positive; 9 for *M. tuberculosis*, 1 for *M. avium*, and 1 for *M. xenopi*. For one additional patient, the material originated from the site where, 3 weeks previously, a BCG vaccination had been administered. These 26 samples among the 72 samples with positive *Mycobacterium*-specific probe results but negative culture results were considered to have true-positive results, and the remaining 46 were considered to have false-positive results. In summary, of the 130 specimens with a positive *Mycobacterium*-specific probe result, 84 were positive and 46 were negative. Of the 682 specimens with a negative *Mycobacterium*-specific probe result, 23 were positive and 659 were negative. This gives a sensitivity of 78.5% and a specificity of 93.5% for the *Mycobacterium*-specific probe.

DISCUSSION

MOTT can be encountered throughout the environment, for example, in water (3, 36), and pseudoepidemics with atypical mycobacteria have been described (28). The presence of nonclinically important mycobacteria or free DNA from these organisms can lead to false-positive results that are not clinically significant when a genus-specific probe is used. Steps taken to remove DNA from sterile bronchoscopes and gastroscopes have recently been demonstrated to be useful in reducing false-positive PCR results, which emphasizes the importance of endoscope cleaning with respect to the validation of PCR assay results in general (16, 24). However, pretesting of specimens for mycobacterial DNA to the genus level could be of great advantage, despite the increased concomitant risk of detecting environmental or nonviable mycobacteria.

The findings of this study showed an overall specificity of 93.5% for the *Mycobacterium*-specific probe under study. The false-positive rate (6.5%) can be considered very low, especially since in this calculation clinical signs were not taken into account. This level of specificity is sufficiently high to warrant use of the probe for preliminary screening of all specimens arriving in the laboratory for PCR analysis. The observation that no sample testing positive with the *M. tuberculosis*- or *M. avium*-specific probe tested negative with the *Mycobacterium*-specific probe supports the premise that pretesting to the genus level would not miss an infection that would otherwise be detected by the species-specific probes and is a feasible procedure for a routine laboratory.

Not every sample which had a positive *Mycobacterium*-specific probe result and which was later found by culture to be infected with *M. tuberculosis* or *M. avium* was positive with the respective species-specific probes. Two different explanations for this observation are possible. The genus-specific probe may be more sensitive than the species-specific probes, or it may be that the mycobacterial DNA detected by the genus-specific probe is not from the cultured *M. tuberculosis* or *M. avium*

TABLE 3. PCR results for the 81 specimens showing positive culture

Mycobacterial species	No. of specimens with the indicated result with the following probe:					
	Mycobacterium-specific probe		*M. tuberculosis*-specific probe		*M. avium*-specific probe	
	Positive	Negative	Positive	Negative	Positive	Negative
M. tuberculosis (n = 61)	43	18	37	24	0	61
M. avium (n = 12)	9	3	0	12	7	5
Other mycobacteria (n = 8)[a]	6	2	0	8	0	8

[a] *M. xenopi* (n = 5), *M. marinum* (n = 1), *M. gordonae* (n = 1), and a *Mycobacterium* sp. (n = 1).

organism but rather is from nonculturable mycobacteria or mycobacterial DNA present in the specimen.

Inhibition of PCR amplification proved to be an infrequent occurrence in the current study, at least with respiratory specimens. Of all respiratory specimens tested, only 2.2% inhibited amplification, in contrast to levels of inhibition of 5.3% (excluding stool) or 14.2% (including stool) for nonrespiratory specimens. The rate of inhibition of amplification observed in this study is very low and is in contrast to other published data (18), which indicated a much higher frequency of inhibition. The method of primary specimen decontamination may have an influence on the rate of inhibition. According to our results, determination of inhibition is especially important for nonrespiratory specimens in order to be certain that negative results are not due to the presence of amplification inhibitors in the sample.

In summary, this study showed that pretesting of clinical specimens for mycobacteria to the genus level with a *Mycobacterium*-specific probe offers the routine clinical laboratory the possibility of detecting tuberculous and nontuberculous mycobacteria with one test. Specimens that are positive with the *Mycobacterium*-specific probe can then be identified by hybridizing the products from the same amplification reaction to species-specific probes. The specificity of the genus-specific probe is suitable for a routine laboratory, and all specimens found to be positive with the *M. tuberculosis*- and *M. avium*-specific probes were also found to be positive with the *Mycobacterium*-specific probe.

REFERENCES

1. **Bloom, B. R., and C. J. L. Murray.** 1992. Tuberculosis: commentary on a reemergent killer. Science **257:**1055–1063.
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. **Collins, C. H., J. M. Grange, and M. D. Yates.** 1984. Mycobacteria in water. J. Appl. Bacteriol. **57:**193–211.
4. **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.
5. **D'Amato, R. F., A. A. Wallmann, L. H. Hochstein, P. M. Colanino, M. Scardamaglia, E. Ardila, M. Ghouri, K. Kim, R. C. Patel, and A. Miller.** 1995. Rapid diagnosis of pulmonary tuberculosis using Roche Amplicor *Mycobacterium tuberculosis* PCR test. J. Clin. Microbiol. **33:**2906–2908.
6. **Del Portillo, P., L. A. Murillo, and M. E. Patarroyo.** 1991. Amplification of a species-specific DNA fragment of *Mycobacterium tuberculosis* and its possible use in diagnosis. J. Clin. Microbiol. **291:**2163–2168.
7. **De Wit, D., L. Steyn, S. Shoemaker, and M. Sogin.** 1990. Direct detection of *Mycobacterium tuberculosis* in clinical specimens by DNA amplification. J. Clin. Microbiol. **28:**2437–2441.
8. **Ehler, S., M. Primann, W. Zaki, and H. Hahn.** 1994. Evaluation of a commercial rRNA target amplification assay for detection of *Mycobacterium tuberculosis* complex in respiratory specimens. Eur. J. Clin. Microbiol. Infect. Dis. **13:**827–829.
9. **Eisenach, K. D., M. D. Cave, J. H. Bates, and J. T. Crawford.** 1990. Polymerase chain reaction amplification of a repetitive DNA sequence specific for *Mycobacterium tuberculosis*. J. Infect. Dis. **161:**977–981.
10. **Fries, J. W. U., R. J. Patel, W. F. Plessen, and D. F. Wirth.** 1990. Genus- and species-specific DNA probes to identify mycobacteria using the polymerase chain reaction. Mol. Cell. Probes **4:**87–105.
11. **Glennon, M., T. Smith, M. Cormican, D. Noone, T. Barry, M. Maher, M. Dawson, J. J. Gilmartin, and F. Gannon.** 1994. The ribosomal intergenic spacer region: a target for the PCR based diagnosis of tuberculosis. Tubercle Lung Dis. **75:**353–360.
12. **Hance, A. J., B. Grandchamp, V. Lévy-Frébault, D. Lecossier, J. Rauzier, D. Bocart, and B. Gicquel.** 1989. Detection and identification of mycobacteria by amplification of mycobacterial DNA. Mol. Microbiol. **3:**843–849.
13. **Hermans, P. W. M., A. R. J. Schuitema, D. Van 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.
14. **Horsburgh, C. R.** 1991. *Mycobacterium avium* complex infection in the acquired immunodeficiency syndrome. N. Engl. J. Med. **324:**1332–1338.
15. **Horsburgh, C. R., and A. Pozniak.** 1993. Epidemiology of tuberculosis in the era of HIV. AIDS **7**(Suppl. 1)**:**109–114.
16. **Kaul, K., S. Luke, C. McGuren, N. Snowden, C. Monti, and W. A. Fry.** 1996. Amplification of residual DNA sequences in sterile bronchoscopes leading to false positive PCR results. J. Clin. Microbiol. **34:**1949–1951.
17. **Kent, P. T., and G. Kubica.** 1985. Public health mycobacteriology. A guide for the level III laboratory. Centers for Disease Control, Atlanta, Ga.
18. **Kirschner, P., J. Rosenau, B. Springer, K. Teschner, K. Feldmann, and E. C. Böttger.** 1996. Diagnosis of mycobacterial infections by nucleic acid amplification: 18-month prospective study. J. Clin. Microbiol. **34:**304–312.
19. **Loeffelholz, M. J., C. A. Lewinski, S. R. Silver, A. P. Purohit, S. A. Herman, D. A. Buonagurio, and E. A. Dragon.** 1992. Detection of *Chlamydia trachomatis* in endocervical specimens by polymerase chain reaction. J. Clin. Microbiol. **30:**2847–2851.
20. **Mustafa, A. S., A. Ahmed, A. T. Abdal, and T. D. Chugh.** 1995. Establishment and evaluation of a multiplex polymerase chain reaction for detection of mycobacteria and specific identification of *Mycobacterium tuberculosis* complex. Tubercle Lung Dis. **76:**336–343.
21. **Patel, R. J., J. T. Kvach, and P. Mounts.** 1986. Isolation and restriction endonuclease analysis of mycobacterial DNA. J. Gen. Microbiol. **132:**541–551.
22. **Pfyffer, G. E., P. Kissling, E. M. I. Jahn, H. M. Welscher, M. Salfinger, and R. Weber.** 1996. Diagnostic performance of amplified *Mycobacterium tuberculosis* direct test with cerebrospinal fluid, other non-respiratory, and respiratory specimens. J. Clin. Microbiol. **34:**834–841.
23. **Romanus, V., H. O. Hollander, P. Whalen, A. M. Olinder-Nielsen, P. H. W. Magnusson, and N. L. Juhlin.** 1995. Atypical mycobacteria in extrapulmonary disease among children. Incidence in Sweden from 1969 to 1990, related to changing BCG-vaccination coverage. Tubercle Lung Dis. **76:**300–310.
24. **Roosendaal, R., E. J. Kuipers, A. J. C. van den Brule, A. S. Peña, A. M. Uyterlinde, J. M. M. Walboomers, S. G. M. Meuwissen, and J. de Graaff.** 1994. Importance of the fiberoptic endoscope cleaning procedure for detection of *Helicobacter pylori* in gastric biopsy specimens by PCR. J. Clin. Microbiol. **32:**1123–1126.
25. **Shafer, R. W., and M. F. Sierra.** 1992. *Mycobacterium xenopi*, *Mycobacterium fortuitum*, *Mycobacterium kansasii*, and other non-tuberculous mycobacteria in an area of endemicity for AIDS. Clin. Infect. Dis. **15:**161–162.
26. **Shankar, P., N. Manjunath, K. K. Mohan, K. Praasad, M. Behari, G. K. Shriniwas, and K. Ahuja.** 1991. Rapid diagnosis of tuberculous meningitis by polymerase chain reaction. Lancet **337:**5–7.
27. **Sjöbring, U., M. Meckelburg, A. B. Anderses, and H. Miörner.** 1990. Polymerase chain reaction for detection of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **28:**2200–2204.
28. **Sniadack, D. H., S. M. Ostroff, M. A. Karlix, R. W. Smithwick, B. Schwartz, M. A. Sprauer, V. A. Silcox, and R. C. Good.** 1993. A nosocomial pseudooutbreak of *Mycobacterium xenopi* due to a contaminated potable water supply: lessons in prevention. Infect. Control Hosp. Epidemiol. **14:**636–641.
29. **Sritharan, V., and R. H. J. Barker.** 1991. A simple method for diagnosing *M. tuberculosis* infection in clinical samples using PCR. Mol. Cell. Probes **5:**385–395.
30. **Stauffer, F., R. Mutschlechner, P. Hasenberger, S. Stadlbauer, and H. Schinko.** 1995. Detection of *Mycobacterium tuberculosis* complex in clinical specimens by a commercial polymerase chain reaction kit. Eur. J. Clin. Microbiol. Infect. Dis. **14:**1046–1051.
31. **Sudre, P., B. J. Hirschel, J. M. Gatell, S. Schwander, S. Vella, C. Katlama, B. Ledergerber, A. d'Aminio Monforte, F. D. Goebel, P. O. Pehrson, C. Pedersen, J. D. Lungren, and the AIDS in Europe Study Group.** 1996. Tuberculosis among European patients with the acquired immune deficiency syndrome. Tubercle Lung Dis. **77:**322–328.
32. **Telenti, A., F. Marchesi, M. Balz, F. Bally, E. C. Böttger, and T. Bodmer.** 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. **31:**175–178.
33. **Tevere, V. J., P. L. Hewitt, A. Dare, P. Hocknell, A. Keen, J. P. Spadoro, and K. K. Y. Young.** 1996. Detection of *Mycobacterium tuberculosis* by PCR amplification with pan-*Mycobacterium* primers and hybridization to an *M. tuberculosis*-specific probe. J. Clin. Microbiol. **34:**918–923.
34. **Weber, J., T. Mettang, E. Staerz, C. Machleidt, and U. Kuhlmann.** 1989. Pulmonary disease due to *Mycobacterium xenopi* in a renal allograft recipient: report of a case and review. Rev. Infect. Dis. **11:**964–969.
35. **Wolinsky, E.** 1979. Non-tuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. **119:**107–159.
36. **Wright, E. P., C. H. Collins, and M. D. Yates.** 1985. *Mycobacterium xenopi* and *Mycobacterium kansasii* in a hospital water supply. J. Hosp. Infect. **6:** 175–178.