JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1999, p. 175–178 Vol. 37, No. 1
0095-1137/99/$04.00+0

False-Positive Gen-Probe Direct *Mycobacterium tuberculosis* Amplification Test Results for Patients with Pulmonary *M. kansasii* and *M. avium* Infections

JAMES H. JORGENSEN,[1,2]* JESSE R. SALINAS,[3] ROSEMARY PAXSON,[3] KAREN MAGNON,[4] JAN E. PATTERSON,[2] AND THOMAS F. PATTERSON[2]

Departments of Pathology[1] and Medicine,[2] The University of Texas Health Science Center, San Antonio, Texas 78284; Microbiology Laboratory, University Hospital, San Antonio, Texas 78229[3]; and Baptist Health System, San Antonio, Texas 78205[4]

Received 16 July 1998/Returned for modification 22 September 1998/Accepted 6 October 1998

The Gen-Probe Amplified Mycobacterium Tuberculosis Direct (MTD) test has been approved for use in the United States for the rapid diagnosis of pulmonary tuberculosis in patients with acid-fast smear-positive sputum samples since 1996. Four patients infected with human immunodeficiency virus and one chronic pulmonary-disease patient seen in our institutions with abnormal chest radiographs and fluorochrome stain-positive sputa were evaluated for tuberculosis, including performance of the MTD test on expectorated sputum samples. Three of these five patients' sputa were highly smear-positive (i.e., more than 100 bacilli per high-power field), while two patient's sputa contained 1 to 10 bacilli per field. MTD results on sputum specimens from these patients ranged from 43,498 to 193,858 relative light units (RLU). Gen-Probe has defined values of at least 30,000 RLU as indicative of a positive test, i.e., the presence of *Mycobacterium tuberculosis* RNA. Four of the patients' sputum cultures yielded growth of *M. kansasii* within 6 to 12 days, and the fifth produced growth of *M. avium* only. One patient's culture contained both *M. kansasii* and *M. avium*, but none of the initial or follow-up cultures from these five patients revealed *M. tuberculosis*. However, subsequent cultures from three of the patients again revealed *M. kansasii*. During the period of this study, in which MTD tests were performed on smear-positive sputum specimens from 82 patients, four of seven patients with culture-proven *M. kansasii* pulmonary infections yielded one or more false-positive MTD tests. The MTD sensitivity observed in this study was 93.8%, and the specificity was 85.3%. Five cultures of *M. kansasii* (including three of these patients' isolates and *M. kansasii* ATCC 12478), and cultures of several other species were examined at densities of 10^5 to 10^7 viable CFU/ml by the MTD test. All five isolates of *M. kansasii* and three of three isolates of *M. simiae* yielded false-positive test results, with readings of 75,191 to 335,591 RLU. These findings indicate that low-level false-positive MTD results can occur due to the presence of *M. kansasii*, *M. avium*, and possibly other *Mycobacterium* species other than *M. tuberculosis* in sputum. Low-level positive MTD results of 30,000 to 500,000 RLU should be interpreted in light of these findings. It remains to be determined if the enhanced MTD test (MTD 2) recently released by Gen-Probe will provide greater specificity than that observed in this report with its first-generation test.

The incidence of tuberculosis in the United States increased by 20% from 1985 to 1992 (8). Outbreaks of multidrug-resistant *Mycobacterium tuberculosis* raised the awareness of health care workers and the general public regarding the reemerging threat of tuberculosis (8, 10, 11). Both clinical and public health laboratories were called upon to provide faster means of definitive diagnosis of tuberculosis and to provide drug susceptibility results for all isolates (26, 27). The Centers for Disease Control and Prevention have recommended that clinical laboratories employ the most rapid methods available, including nucleic acid probes for identification of positive mycobacterial cultures, and that they consider even faster direct nucleic acid amplification techniques for direct detection of *M. tuberculosis* (9, 26). Two commercial methods for amplification of nucleic acids directly from acid-fast smear-positive respiratory specimens have been approved for use in the United States by the Food and Drug Administration (FDA). The first was the transcription-mediated amplification test (Amplified Mycobacterium Tuberculosis Direct [MTD] test) marketed by Gen-Probe (San Diego, Calif.), followed later by the Amplicor PCR assay (Roche Molecular Systems, Branchburg, N.J.) (9). The present communication describes our experience with several false-positive MTD test results due to *M. kansasii* and *M. avium* infections in patients with AIDS and in one human immunodeficiency virus (HIV)-negative patient with chronic lung disease due to *M. avium*.

* Corresponding author. Mailing address: Department of Pathology, University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78284-7750. Phone: (210) 567-4088. Fax: (210) 567-2367. E-mail: jorgensen@uthscsa.edu.

MATERIALS AND METHODS

Patients. All but one of the patients included in this study were in the advanced stage of AIDS and were suspected of having pulmonary tuberculosis. Salient features of each case are summarized as follows.

Patient 1 is a 38-year-old HIV-positive male who was undergoing treatment for osteomyelitis due to *Pseudomonas aeruginosa*. He was admitted due to increased low-back pain, low-grade fevers, and a cough productive of yellow-green sputum for 1 month. A chest radiograph indicated a right lower-lobe perihilar infiltrate. A sputum specimen revealed the presence of fluorochrome-positive bacilli graded as 4+. The Gen-Probe MTD test yielded a low-positive luminescence value of 53,680 relative light units (RLU). On that basis, the patient was started on standard four-drug antituberculosis therapy (i.e., isoniazid, rifampin, ethambutol, and pyrazinamide). A repeat sputum specimen 20 days later revealed 2+ fluorochrome-positive bacilli. However, a repeat MTD test on that specimen was negative (8,414 RLU). Suspicion that his MTD test results were falsely positive led to discontinuation of pyrazinamide, and elevated liver function test values led to discontinuation of his isoniazid therapy. Rifampin was also stopped after

TABLE 1. Initial Gen-Probe MTD, smear, and culture results for five patients suspected of having tuberculosis

Patient no. (description)[a]	Result of:		
	Fluorochrome-stained sputum smear[b]	MTD test on sputum (RLU)[c]	Sputum culture
1 (38, male, AIDS)	4+	53,680	Heavy growth; *M. kansasii* and *M. avium* complex
2 (26, male, AIDS)	4+	193,858	Heavy growth; *M. kansasii*
3 (44, female, AIDS)			
Specimen 1	1+	11,750	*M. kansasii*
Specimen 2	2+	43,498	*M. kansasii*
4 (31, female, AIDS)	4+	87,237	*M. kansasii*
5 (63, male, HIV-negative)	2+	193,690	*M. avium*

[a] Description includes age in years, sex, and HIV or AIDS status.
[b] Graded degree of smear positivity.
[c] Read on a luminometer; a positive test result was defined in the Gen-Probe package insert as ≥30,000 RLU.

several more days because of persistent liver function test abnormalities. The patient was discharged for hospice care after 34 days because of further complications. His sputum cultures yielded growth of both *M. kansasii* and *M. avium* but no evidence of *M. tuberculosis*.

Patient 2, a 26-year-old African-American male with AIDS and a previous history of *M. kansasii* pulmonary disease and noncompliance with his prescribed course of therapy for that condition, presented with fever, cough, and pleuritic chest pain. Chest radiographs revealed diffuse perihilar infiltrates. He was admitted for evaluation and workup for possible tuberculosis. A sputum fluorochrome stain was 4+ and was followed by a positive MTD test result (193,858 RLU). The patient was started on standard four-drug antituberculosis therapy and remained in the hospital in respiratory isolation. When the patient's culture became positive for *M. kansasii*, and *M. tuberculosis* was not detected, he was discharged on therapy for *M. kansasii* (i.e., isoniazid, ethambutol, and rifampin).

Patient 3, a 44-year-old woman with AIDS, was seen in the outpatient department for possible respiratory infection, including the possibility of tuberculosis or *M. avium* infection. Two sputum specimens collected on different days were submitted to the laboratory and processed separately. One specimen contained 1+ fluorochrome-positive bacilli, and the other was interpreted as a 2+ smear. The first sample yielded a negative MTD test result (11,750 RLU), while the second specimen was regarded as positive, with a reading of 43,498 RLU. Because of the recent experience with patients 1 and 2, she was not immediately started on antituberculosis medications. Her culture was positive for *M. kansasii* only.

Patient 4, a 31-year-old Caucasian female, presented to the Emergency Department with a 1-month history of worsening cough and congestion, low-grade temperature, night sweats, and moderate weight loss. A chest radiograph revealed bilateral upper-lobe infiltrates. The patient was admitted for evaluation of pneumonia or possible active tuberculosis and was placed in respiratory isolation. An HIV antibody screen was positive and was confirmed by Western blot, with an absolute $CD4^+$ cell count subsequently determined to be $34/mm^3$. Two sputum specimens collected on consecutive days revealed the presence of 4+ fluorochrome-positive bacilli. The MTD test performed on one of the specimens yielded a reading of 87,237 RLU. On the basis of these results, the patient was placed on four-drug antituberculosis therapy, (i.e., isoniazid, rifampin, ethambutol, and pyrazinamide). A tuberculin skin test placed at the time of admission was negative. On hospital day 3, both sputum cultures revealed growth of an acid-fast organism by use of the BACTEC system (Becton-Dickinson Microbiology Systems, Cockeysville, Md.); this organism was identified as *M. kansasii* by the Accu-Probe test (Gen-Probe). Pyrazinamide was discontinued, and the patient was discharged on three-drug therapy (i.e., isoniazid, ethambutol, and rifampin).

Patient 5 is a 63-year-old HIV-negative Caucasian male with a history of treatment 7 years earlier for *M. avium* pulmonary disease as a result of heavy tobacco use since the age of 8. At the time of his original infection, he had a productive cough with hemoptysis, fever, night sweats, and weight loss, and *M. avium* was isolated from his sputum. He was treated with a combination of antimicrobial agents for at least 6 months. Approximately 5 months before the present admission, he was diagnosed with squamous-cell carcinoma of the base of the tongue that also involved the larynx. He received both radiation and chemotherapy during the following several months. Upon readmission for follow-up endoscopy and biopsy, he was noted to have had a recent 8-lb weight loss, a productive cough with thick sputum, and multiple pulmonary nodules on radiograph and CT scan consistent with either metastasis of his tumor or recurrence of his mycobacterial disease. Sputum samples contained 2+ fluorochrome-positive bacilli and yielded a low-level-positive MTD test result (193,690 RLU). His sputum cultures yielded growth of *M. avium* only.

Mycobacterial culture procedures. Specimens were initially processed in one of the three laboratories by using the *N*-acetyl-L-cysteine NaOH procedure for digestion and decontamination (21). Fluorochrome smears were prepared from the sputum concentrates with the auramine-rhodamine stain (21). Concentrated sputum sediment was cultured with either a manual broth culture system, Septi-Chek or MGIT (Becton Dickinson Microbiology Systems), used according to the manufacturer's instructions, or an instrument broth culture system, the BACTEC radiometric or the MB BACT system (Organon Teknika, Durham, N.C.). The laboratories also inoculated either Middlebrook and Cohn 7H11 or Löwenstein-Jensen solid culture medium. Mycobacterial cultures were incubated for 4 to 6 weeks, depending on the individual laboratory's policy. Mycobacterial isolates were identified by use of the Accu-Probe (Gen-Probe) method according to the manufacturer's guidelines with probes for *M. tuberculosis* complex, *M. avium* complex, and *M. kansasii*. Broth cultures were probed for the presence of *M. tuberculosis* even if they were positive for *M. kansasii* or *M. avium* complex. In addition, solid culture media were incubated for an extended period and examined for the presence of multiple mycobacterial species.

Performance of the Gen-Probe MTD test. The direct Gen-Probe MTD test was performed in one of the laboratories (University Hospital) by following the manufacturer's procedures explicitly (13). All tests were performed by the same microbiologist, who had undergone training at the product manufacturer's site and was subsequently certified by Gen-Probe to perform the test on clinical samples. MTD tests were read in a Gen-Probe Leader 50 luminometer at the end of the probe selection step. Each MTD test run included positive and negative amplification controls and hybridization controls, and in addition, each patient's sputum sample was tested for potential MTD test inhibition by spiking a portion of the sample with the *M. tuberculosis* positive-control suspension. A positive MTD test result was defined by Gen-Probe as a luminescence reading of 30,000 RLU or greater. Cultures of several *Mycobacterium* species were tested by suspending the growth of patients' isolates or control strains in sterile 0.9% saline to the density of a McFarland opacity standard of 1. These suspensions were then used in place of sputum concentrate in the standard MTD test procedure and were tested at the standard 1:100 dilution specified in the package insert (called dilution 1). Colony counts of each of the mycobacterial culture suspensions tested in this manner were performed on 7H11 agar. Multiple MTD test kits with different lot numbers were used for the direct tests on patients' sputum samples and for the culture suspension tests described above.

RESULTS

This report describes low-level-positive MTD test results from five patients' smear-positive sputum specimens that contained either *M. kansasii* or *M. avium* but were not found to contain *M. tuberculosis*. The MTD results of the patients' specimens ranged from 43,498 to 193,858 RLU (Table 1). Three of five specimens contained large numbers of acid-fast bacilli based upon the microscopic examination of fluorochrome-stained smears of concentrated sputum. However, two patients' specimens contained only 1 to 10 bacilli per high-power field. In one case an initial MTD test gave a negative result for a sputum specimen that contained only 1+ acid-fast bacilli based on examination of the fluorochrome smear, while a second specimen obtained 2 days later contained a slightly larger number of acid-fast bacilli (2+) and gave a low-level-positive MTD test result (Table 1). Patient 2 had a follow-up culture that was still positive for *M. kansasii* 10 months later, but the MTD test result for that specimen was negative.

The initial findings with the MTD test performed directly on sputum specimens from these patients led us to examine cultures of several mycobacterial species. When culture-grown suspensions of stock culture or of patient isolates of *M. kansasii*

TABLE 2. MTD test results on saline suspensions of mycobacteria[a] prepared from cultures and diluted 1:100

Isolate	Approx concn (CFU/ml) tested[b]	MTD test result (RLU)
M. tuberculosis ATCC 25177	6×10^5	3,136,425
M. kansasii ATCC 12478	3×10^6	204,579
M. kansasii from patient 1	1.4×10^7	193,073
M. kansasii from patient 2	6×10^6	187,642
M. kansasii from patient 3	1×10^5	114,861
M. simiae 2170	3×10^6	195,009
M. simiae 1389	3×10^6	117,228
M. avium UH	3×10^6	13,086
M. fortuitum 977	ND	6,652
M. chelonae 1910	ND	13,447
M. szulgai 870	ND	7,508
M. xenopi 729	ND	9,103

[a] At the density of a McFarland opacity standard of 1.
[b] ND, not determined.

and *M. simiae* were tested at densities of $\geq 10^5$ CFU/ml, MTD test results of >100,000 RLU were obtained. However, culture suspensions of several other mycobacteria other than *M. tuberculosis* (MOTT) did not give rise to positive MTD test results, even at cell densities exceeding 10^7 CFU/ml (Table 2).

Our laboratory (University Hospital) has performed MTD tests on smear-positive sputum specimens from a total of 82 separate patients since the test was incorporated into our standard procedure in 1996 (data not shown). Of the 48 patients whose specimens were culture-positive for *M. tuberculosis*, 43 have had MTD test results of >1,000,000 RLU (test sensitivity = 89.6%). Only two marginally smear-positive specimens that were culture-positive for *M. tuberculosis* yielded MTD test results lower than this number, specifically, 157,824 and 588,343 RLU. If these two specimens are included as positive, the overall sensitivity of the test in our series is 93.8%. There have been five false-positive results, those described herein, during this period, resulting in a test specificity of 85.3% in our experience. There have been 19 other patients with culture-documented MOTT infections whose MTD test results were lower than 30,000 RLU. These included 3 other patients infected with *M. kansasii*, 10 with *M. avium*, 4 with *M. simiae*, 1 with *M. chelonae*, and 1 with *M. gordonae*.

DISCUSSION

The Centers for Disease Control and Prevention and many state, territorial, and local public health departments have focused considerable energy and resources on controlling the reemergence of tuberculosis that was noted to occur in the United States between the mid-1980s and 1992 (8, 10, 11, 27, 30). Their efforts have resulted in a 26% decrease in the number of cases reported between 1992 and 1997 (11). Contributing to the reversal of this trend have been measures such as improved case contact tracing, prompt initiation of appropriate antituberculosis therapy with four drugs, including directly observed therapy, and laboratory measures that allow for the prompt identification of persons with tuberculosis (10, 11). The resurgence of tuberculosis has provided a strong impetus to increase the efficiency and rapidity of laboratory methods for the detection of *M. tuberculosis*. The use of the fluorochrome stain, prompt performance of smears and prompt reporting of smear results, faster methods for the isolation of mycobacteria (i.e., use of broth culture media), rapid identification of isolates by nucleic acid probes, and faster drug susceptibility testing procedures (e.g., the radiometric method) have all contributed to an overall sharp reduction in the time required to document infection with *M. tuberculosis* (27, 30). However, nucleic amplification methods for direct testing of smear-positive sputum specimens, now available, represent the most rapid laboratory methods for the documentation of *M. tuberculosis* infection (9).

By use of the Gen-Probe MTD or Roche Amplicor amplification method, it is possible to determine within 8 h of specimen collection whether an acid-fast organism seen in a patient's sputum is *M. tuberculosis* (1, 3, 12, 16, 19, 23, 25). The MTD test was approved by the FDA and has been actively marketed in the United States starting in January 1996. The test amplifies 16S rRNA of *Mycobacterium* species by transcription-mediated amplification (2, 18). The resulting amplicons are then detected by a hybridization protection assay using a probe that purportedly is specific for *M. tuberculosis* (13, 18).

Shortly after initiating use of the MTD test in our setting, we began to experience the false-positive results described above. Four of the five patients with false-positive results had AIDS and were suspected of having pulmonary tuberculosis. Only patients 1 and 5 were known to have had previous infections with a MOTT, i.e., *M. avium*. None of the patients had a history of tuberculosis or were undergoing treatment for tuberculosis, which might lead to persistence of *M. tuberculosis* rRNA in sputum (20). Despite attempts to isolate *M. tuberculosis* from these patients' specimens and despite examination of their cultures by the Accu-Probe test, there was no evidence of concurrent infection with *M. tuberculosis* in addition to *M. kansasii* or *M. avium*. One patient's culture contained both *M. kansasii* and *M. avium*. However, subsequent cultures from three of the patients again revealed *M. kansasii*. During this period, a total of seven patients examined by us had acid-fast smear-positive sputum specimens due to *M. kansasii* (data not shown). Four of the seven patients had false-positive MTD test results, as described above. Furthermore, direct examination of the cultures of *M. kansasii* from our first three patients and of a type strain control culture provided evidence of false-positive MTD test readings due to the presence of that species and, in addition, of *M. simiae*.

Our findings now extend the list of *Mycobacterium* species that can potentially lead to false-positive MTD tests. *M. celatum* has been reported previously to represent a possible source of false-positive probe results (4) because strains of this species differ from *M. tuberculosis* by only 1 to 2 bp in the ~20-bp target sequence for the probe used by Gen-Probe for amplicon detection (5). Indeed, there have been several reports of infection with *M. celatum* in HIV-infected (3, 24), and immunocompetent patients (6). However, *M. kansasii* and *M. simiae* are more commonly encountered in specimens from immunocompromised patients (7, 22, 28, 29); they differ by only 4 bp from *M. tuberculosis* in the MTD probe region (2). In fact, *M. gastri*, *M. scrofulaceum*, *M. kansasii*, and *M. simiae* are all identical in the probe region of the 16S rRNA (2). However, false-positive probe results with *M. avium* complex isolates are unexpected because they have even fewer base pairs in common with *M. tuberculosis* in the probe region (2). Despite this difference, ours is the second report of false-positive MTD test results apparently due to infection with *M. avium* (17).

The findings described herein for the first two of our patients led to a revision of the MTD test package insert in August 1996. The possibility of false-positive results due to large numbers of *M. kansasii* organisms was included in the revised MTD test package insert (14). Gen-Probe then advised that readings of 30,000 to 500,000 RLU represented a low range of positivity that could be the result of large numbers of MOTT. It was recommended that test results in this range be viewed as "inconclusive" (14). However, our data do not entirely support

the concept that only very high densities of MOTT can lead to low-level-positive results. In fact, our findings are in contrast with the statement in the package insert that the MTD test will not cross-react when fewer than 2×10^6 to 4×10^6 CFU per test are present (14). Moreover, two of our five patients had only 2+ acid-fast bacilli observed on their concentrated sputum fluorochrome smears. Only two specimens in our series that were culture-positive for *M. tuberculosis* yielded MTD readings lower than 1,000,000 RLU.

It is our belief that the Gen-Probe MTD test has substantial value in the early diagnosis of tuberculosis in patients with acid-fast smear-positive sputum specimens. However, we believe that MTD test results in the range of 30,000 to 500,000 RLU should be interpreted with caution but that readings higher than 500,000 RLU can be considered indicative of the presence of *M. tuberculosis*. This may be further refined by knowledge of the degree of smear positivity; i.e., low-level-positive MTD results from sputum specimens with 2+ to 4+ acid-fast bacilli probably represent false-positive results, whereas low-level-positive MTD results from sputa with scant numbers of bacilli may represent true-positive results for *M. tuberculosis*.

In our limited series, the sensitivity of the MTD test was 93.8% and the specificity was 85.3% when a positive test result was defined as ≥30,000 RLU. Our findings regarding the sensitivity of the MTD test are quite similar to those of previous investigators, but the specificity of the test observed by us is somewhat lower than that previously reported (1, 12, 16, 18, 19, 23, 24). The lower specificity observed by us may be a function of the number of pulmonary infections due to large numbers of MOTT in the sputa of our patients. Gen-Probe has recently released a second-generation MTD test (referred to as MTD 2) that incorporates several procedural changes, including a larger specimen volume, a shorter amplification phase, and a longer probe selection step (15). These changes may enhance the specificity of the test by limiting the number of amplicons produced and extending the probe selection phase. However, the package insert for the revised procedure still includes the possibility of results in an indeterminate range, which would require repeating the test (15). Thus, it remains to be determined if this "enhanced" MTD 2 test will make our recommendations for MTD test interpretation unnecessary.

ACKNOWLEDGMENT

Vivian Jonas of Gen-Probe provided helpful advice in the conduct of this study and MTD kits for testing of the MOTT cultures.

REFERENCES

1. **Abe, C., K. Hirano, M. Wada, Y. Kazumi, M. Takahashi, Y. Fukasawa, T. Yoshimura, C. Miyagi, and 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. **Boddinghaus, B., T. Rogall, T. Flohr, H. Blocker, and E. C. Bottger.** 1990. Detection and identification of mycobacteria by amplification of rRNA. J. Clin. Microbiol. **28:**1751–1759.
3. **Bonomo, R. A., J. M. Briggs, W. Gross, M. Hassan, R. C. Graham, W. R. Butler, and R. A. Salata.** 1998. *Mycobacterium celatum* infection in a patient with AIDS. Clin. Infect. Dis. **26:**243–244.
4. **Butler, W. R., S. P. O'Connor, M. A. Yakrus, and W. M. Gross.** 1994. Cross-reactivity of genetic probe for detection of *Mycobacterium tuberculosis* with newly described species *Mycobacterium celatum*. J. Clin. Microbiol. **32:**536–538.
5. **Butler, W. R., S. P. O'Connor, M. A. Yakrus, R. W. Smithwick, B. B. Pilikaytis, C. W. Moss, M. M. Floyd, C. L. Woodley, J. O. Kilburn, F. S. Vadney, and W. M. Gross.** 1993. *Mycobacterium celatum* sp. nov. Int. J. Syst. Bacteriol. **43:**539–548.
6. **Bux-Gewehr, I., H. P. Hagen, S. Rüsch-Gerdes, and G. Feurle.** 1998. Fatal pulmonary infection with *Mycobacterium celatum* in an apparently immunocompetent patient. J. Clin. Microbiol. **36:**587–588.
7. **Campo, R. E., and C. E. Campo.** 1997. *Mycobacterium kansasii* disease in patients infected with human immunodeficiency virus. Clin. Infect. Dis. **24:** 1233–1238.
8. **Cantwell, M. F., D. E. Snider, G. M. Cauthen, and I. M. Onorator.** 1994. Epidemiology of tuberculosis in the United States, 1985 through 1992. JAMA **272:**535–539.
9. **Centers for Disease Control and Prevention.** 1996. Nucleic acid amplification tests for tuberculosis. Morbid. Mortal. Weekly Rep. **45:**950–952.
10. **Centers for Disease Control and Prevention.** 1997. Tuberculosis morbidity—United States, 1996. Morbid. Mortal. Weekly Rep. **46:**695–700.
11. **Centers for Disease Control and Prevention.** 1998. Tuberculosis morbidity—United States, 1997. Morbid. Mortal. Weekly Rep. **47:**253–257.
12. **Dalovisio, J. R., S. Montenegro-James, S. A. Kemmerly, C. F. Genre, R. Chambers, D. Greer, G. A. Pankey, D. M. Failla, K. G. Haydel, L. Hutchison, M. F. Lindley, B. M. Nunez, A. Praba, K. D. Eisenach, and E. S. Cooper.** 1996. Comparison of the Amplified *Mycobacterium tuberculosis* (MTB) Direct Test, Amplicor MTB PCR, and IS6110-PCR for detection of MTB in respiratory specimens. Clin. Infect. Dis. **23:**1099–1106.
13. **Gen-Probe.** 1995. Amplified Mycobacterium Tuberculosis Direct Test for in vitro diagnostic use: 50-test kit (package insert). Gen-Probe, San Diego, Calif.
14. **Gen-Probe.** 1996. Amplified Mycobacterium Tuberculosis Direct Test for in vitro diagnostic use: 50-test kit (revised package insert). Gen-Probe, San Diego, Calif.
15. **Gen-Probe.** 1998. Amplified Mycobacterium Tuberculosis Direct Test for in vitro diagnostic use: 50-test kit (revised package insert). Gen-Probe, San Diego, Calif.
16. **Ichiyama, S., Y. Iinuma, Y. Tawada, S. Yamori, Y. Hasegawa, K. Shimokata, and N. Nakashima.** 1996. Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test and Roche PCR-microwell plate hybridization method (AMPLICOR MYCOBACTERIUM) for direct detection of mycobacteria. J. Clin. Microbiol. **34:**130–133.
17. **Javellana, E. D., and M. J. Zervos.** 1998. False-positive Gen-Probe direct amplification test in a case of *Mycobacterium avium* complex infection. Clin. Infect. Dis. **26:**255–256.
18. **Jonas, V., J. J. Alden, J. I. Curry, K. Kamisango, C. A. Knott, R. Lankford, 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.
19. **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.
20. **Moore, D. F., J. I. Curry, C. A. Knott, and V. Jonas.** 1996. Amplification of rRNA for assessment of treatment response of pulmonary tuberculosis patients during antimicrobial therapy. J. Clin. Microbiol. **34:**1745–1749.
21. **Nolte, F. S., and B. Metchock.** 1995. *Mycobacterium*, p. 400–437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
22. **Patel, R., G. D. Roberts, M. R. Keating, and C. V. Paya.** 1994. Infections due to nontuberculous mycobacteria in kidney, heart, and liver transplant recipients. Clin. Infect. Dis. **19:**263–273.
23. **Piersimoni, C., A. Callegaro, D. Nista, S. Bornigia, F. De Conti, G. Santini, and G. De Sio.** 1997. Comparative evaluation of two commercial amplification assays for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. J. Clin. Microbiol. **35:**193–196.
24. **Piersimoni, C., E. Tortoli, F. de Lalla, D. Nista, D. Donato, S. Bornigia, and G. De Sio.** 1997. Isolation of *Mycobacterium celatum* from patients infected with human immunodeficiency virus. Clin. Infect. Dis. **24:**144–147.
25. **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 nonrespiratory, and respiratory specimens. J. Clin. Microbiol. **34:**834–841.
26. **Tenover, F. C., J. T. Crawford, R. E. Huebner, L. J. Geiter, C. R. Horsburgh, Jr., and R. C. Good.** 1993. The resurgence of tuberculosis: is your laboratory ready? J. Clin. Microbiol. **31:**767–770. (Guest commentary.)
27. **Tokars, J. I., J. R. Rudnick, K. Kroc, L. Managan, G. Pugliese, R. E. Huebner, J. Chan, and W. R. Jarvis.** 1996. U.S. hospital mycobacteriology laboratories: status and comparison with state public health department laboratories. J. Clin. Microbiol. **34:**680–685.
28. **Tortoli, E., M. T. Simonetti, C. Lachini, V. Penati, and P. Urbano.** 1994. Tentative evidence of AIDS-associated biotype of *Mycobacterium kansasii*. J. Clin. Microbiol. **32:**1779–1782.
29. **Velainis, G. T., L. M. Cardona, and D. L. Greer.** 1991. The spectrum of *Mycobacterium kansasii* disease associated with HIV-1-infected patients. J. Acquired Immune Defic. Syndr. **4:**516–520.
30. **Woods, G. L., T. A. Long, and F. G. Witebsky.** Mycobacterial testing in clinical laboratories that participate in the College of American Pathologists mycobacteriology surveys: changes in practices based on responses to 1992, 1993, and 1995 questionnaires. Arch. Pathol. Lab. Med. **120:**429–435.