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Use of Gen-Probe AccuProbes To Identify *Mycobacterium avium* Complex, *Mycobacterium tuberculosis* Complex, *Mycobacterium kansasii*, and *Mycobacterium gordonae* Directly from BACTEC TB Broth Cultures

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To evaluate the utility of Gen-Probe AccuProbes for the identification of mycobacteria directly from BACTEC TB 12B vials containing acid-fast bacilli, culture results for 11,375 clinical specimens other than blood received from 1 January 1992 to 30 September 1993 were reviewed retrospectively. During this period, a total of 359 of 11,375 BACTEC vials were positive for acid-fast bacilli and were evaluated for mycobacteria with one or more probes: 224 were probed for *Mycobacterium tuberculosis* complex, 253 were probed for *Mycobacterium avium* complex, 64 were probed for *Mycobacterium kansasii*, and 77 were probed for *Mycobacterium gordonae*. After initial testing with the probes, 75 vials were positive for *M. tuberculosis* complex, 99 were positive for *M. avium* complex, 11 were positive for *M. kansasii*, and 55 were positive for *M. gordonae*. Repeat testing of vials that were initially probe negative or testing of colonies from subcultures of these vials identified an additional 11 *M. tuberculosis*, 27 *M. avium* complex, 1 *M. kansasii*, and 9 *M. gordonae* that were not detected on initial screening. On the basis of these data, the percentage of organisms identified directly from the BACTEC TB 12B vials upon initial screening with each of the four AccuProbes was 87.2% for *M. tuberculosis* complex, 78.6% for *M. avium* complex, 91.7% for *M. kansasii*, and 85.9% for *M. gordonae*.

Historically, the isolation and identification of mycobacteria have been very slow processes, taking up to 6 to 8 weeks for growth and another several weeks to months for identification. Recent advances in techniques, however, have dramatically shortened the times to both detection and identification. The radiometric BACTEC system (Becton Dickinson, Cockeysville, Md.) detects growth most rapidly (1, 4, 9, 14). For the identification of mycobacteria, high-performance liquid chromatography (HPLC) (2), which is used primarily in research and reference laboratories, and analysis with DNA probes (5, 7, 11, 13) provide results in a few hours. Currently, only acridinium-ester-labeled DNA probes (AccuProbes; Gen-Probe Inc., San Diego, Calif.) specific for *Mycobacterium tuberculosis* complex (MTBC), *Mycobacterium avium* complex (MAC) (as well as separate probes for *M. avium* and *Mycobacterium intracellulare*), *Mycobacterium kansasii*, and *Mycobacterium gordonae* are commercially available.

It has been shown that for testing colonies of mycobacteria, AccuProbes are 100% specific, whereas sensitivities have varied for the different species or species complexes: 95.2 to 97.2% for MAC, 100% for MTBC, 44% for *M. kansasii*, and 100% for *M. gordonae* (8, 12). However, few studies have examined the reliabilities of the chemiluminescent DNA probes for testing growth directly from BACTEC TB vials. Evans et al. (6) evaluated the probes specific for MAC and MTBC (also referred to here as MAC and MTBC probes) on 120 positive BACTEC TB cultures (which included 17 MTBC and 102 MAC). Sensitivities were 47% for MTBC and 90% for MAC. Thompson et al. (16) tested the MAC and MTBC probes on 60 positive BACTEC cultures (including 21 MTBC and 19 MAC); the sensitivity of both probes was 100%. Chapin-Robertson et al. (3) tested 98 acid-fast bacillus (AFB)-positive BACTEC cultures (82 MAC, 6 *M. tuberculosis*, 6 *M. gordonae*); in that study, 100% of *M. tuberculosis* and *M. gordonae* and 89% of MAC isolates were detected directly from the BACTEC vial. The specificity of the probes used in all three studies was 100%. A major limitation of the three studies was the small number of *M. tuberculosis* cultures that were evaluated. Moreover, the probe for *M. kansasii* was not evaluated, and to our knowledge there are no published data concerning the reliability of the latter probe for the direct identification of growth in BACTEC vials.

The purpose of our study was to expand upon the findings of the earlier studies by reviewing the data from a larger number of MTBC-positive BACTEC TB cultures and to determine the reliability of the *M. kansasii* AccuProbe for the direct testing of growth from a positive BACTEC TB vial. Upon reviewing the results of the study, we have modified our protocol to achieve a more cost-effective use of probes in our laboratory.

MATERIALS AND METHODS

Specimens and specimen processing. Culture results for all specimens except blood that were received for the culture of AFB at the University of Texas Medical Branch from 1 January 1992 to 30 September 1993 were reviewed in the present study. Specimens were processed daily except Sundays. Specimens from sites contaminated with normal flora were decontaminated and were concentrated prior to inoculation by using NAC-PAC *N*-acetyl-L-cysteine/TB Base Solution (Alpha-Tec Systems, Irvine, Calif.) according to the manufacturer's directions. For each specimen, inoculation of the media was as follows: 0.1 ml of specimen was inoculated onto a Lowenstein-Jensen (LJ) slant and onto each side of a Middlebrook 7H11/7H11 Selective Agar plate (Becton Dickinson Microbiology Systems, Cockeysville, Md.), and 0.5 ml was

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inoculated into a BACTEC 12B bottle (<0.5 ml was inoculated when a smaller volume of specimen was received).

BACTEC 12B bottles were monitored on the BACTEC 460 instrument for microbial growth every 72 h for the first 2 weeks and once per week for the next 4 weeks. When the growth index (GI) reached 100, 0.1 ml of the broth medium was subcultured onto an LJ slant, and Kinyoun acid-fast staining (Remel Laboratories, Lenexa, Kans.) was performed to observe the morphology and the acid-fast characteristics of the bacteria present. Bottles with a GI of between 10 (which is the lowest value considered to be positive for growth according to the manufacturer's directions) and 100 were occasionally processed at the request of the physician. If AFB were present, probe analysis was performed.

No additional identification tests were performed if a probe result was positive and the identification was consistent with the morphology and pigmentation of the colonies recovered on solid media (primary media and subcultures). A second probe test was performed on BACTEC vials when the results of the original tests were negative but within 5,000 relative light units of the positive cutoff value.

Conventional solid media were examined once a week for 8 weeks. Isolates not previously detected in BACTEC cultures were identified with the AccuProbes or, if the probes were negative, conventional biochemicals.

Mycobacterial identification. The DNA probe assay was performed twice per week. Two milliliters of broth from an AFB-positive BACTEC 12B bottle was centrifuged at 3,000 × *g* for 15 min. The supernatant was decanted, and the pellet was used in the probe assay according to the manufacturer's procedure for growth from solid media. The probe tests performed were selected on the basis of the pigmentation of the pellet. Nonpigmented pellets were tested with probes for MTBC and MAC; pigmented pellets were tested for MAC and *M. kansasii*. *M. gordonae* probes were used only when the following observations suggested possible contamination of the cultures with *M. gordonae*: smooth appearance of the growth in the BACTEC bottle, shorter darkly staining AFB on smear, and dark orange pigmentation of the pellet. Single probes or other combinations of probes were occasionally tested on the basis of the patient's history (for example, if previous specimens from the patient were known to have been positive for MTBC, the newly positive culture was probed with MTBC).

For isolates not identified by probes, growth rate, pigmentation with and without exposure to light, and the following biochemical tests were used to identify the organism: niacin, arylsulfatase (3 days and 2 weeks), Tween hydrolysis, urease, nitrate reduction, sodium chloride tolerance, semiquantitative and heat-stable catalase, tellurite reduction, and growth on MacConkey agar without crystal violet (Becton Dickinson). Biochemical tests were performed by standard procedures (10).

RESULTS

A total of 11,375 clinical specimens other than blood were cultured for AFB from 1 January 1992 to 30 September 1993. Of these cultures, 359 BACTEC vials (3.2%) were positive for AFB and were evaluated for mycobacteria by using AccuProbes, as follows: 224 were probed for MTBC, 253 were probed for MAC, 64 were probed for *M. kansasii*, and 77 were probed for *M. gordonae*. Of the vials probed for MTBC, 75 were positive on initial testing, and MTBC was eventually detected in 11 of the 149 vials that were initially MTBC probe negative. Of the vials probed for MAC, 99 were positive when first tested, and MAC was ultimately identified in 27 of the 154 vials that were initially MAC probe negative. Two bottles contained both MAC and MTBC. Of the vials probed for *M. kansasii*, 11 were positive on initial testing, and *M. kansasii* was eventually detected in 1 of the remaining 53 vials that were initially *M. kansasii* probe negative. Of the vials probed for *M. gordonae*, 55 were positive when first tested, and *M. gordonae* was ultimately identified in 9 of the 22 vials that were initially *M. gordonae* probe negative. The proportion of organisms identified with probes upon initial testing of growth from BACTEC vials was 87.2% for MTBC, 78.6% for MAC, 91.7% for *M. kansasii*, and 85.9% for *M. gordonae*. These results are summarized in Table 1. For vials that were positive on initial testing, the GI ranged from 18 to >999. The GI values of vials that gave a false-negative result on initial testing ranged from 108 to >999. Because BACTEC vials were batched and tested by probe analysis twice per week, the GI values reported here are minimum values only.

TABLE 1. Proportion of mycobacteria initially identified from AFB-positive BACTEC vials using AccuProbes

Probe tested	No. of vials tested	No. of vials initially positive	Total no. of vials positive	% initially identified[a]
MTBC	224	75	86	87.2
MAC	253	99	126	78.6
M. kansasii	64	11	12	91.7
M. gordonae	77	55	64	85.9

[a] Percent initially identified = number of BACTEC cultures initially positive/total number of cultures positive.

Colonies from subcultures of all probe-positive vials had morphologies consistent with the probe identification. Given the 100% specificity of GenProbe AccuProbes for identifying mycobacteria colonies and growth from BACTEC vials found in previous studies (3, 6, 8, 12, 16), no further identification was performed on these cultures.

An additional 1 MTBC, 11 MAC, 14 *M. kansasii*, and 34 *M. gordonae* were isolated from BACTEC vials that were initially tested with probes for mycobacteria species other than those which were eventually recovered. Since the incorrect probe was chosen for the initial test, results for these vials were not considered false negative and were not included in calculating the percentages presented in Table 1.

Eleven of the 359 vials evaluated contained mycobacteria other than those identifiable by probes: 1 *Mycobacterium xenopi*, 3 *Mycobacterium fortuitum*, 2 *Mycobacterium scrofulaceum*, 3 *Mycobacterium marinum*, 2 *Mycobacterium haemophilum*, and 2 *Mycobacterium chelonae* (*M. haemophilum* and *M. chelonae* were cultured from the same patient's specimen on two separate occasions). A *Rhodococcus* species was recovered from 2 of the 359 AFB smear-positive bottles.

DISCUSSION

Given the recent resurgence of tuberculosis in the United States, it has become increasingly important to isolate and identify mycobacteria, especially MTBC, as rapidly as possible. In an effort to combat the increased incidence of tuberculosis, the Centers for Disease Control and Prevention has made the following recommendations for mycobacterial testing (15): (i) the results of AFB smears should be reported within 1 day of receipt of the specimen; (ii) for isolation, a liquid medium and a solid medium should be inoculated, and the culture system should detect growth within 14 days of specimen receipt; and (iii) DNA probes, the BACTEC NAP (*p*-nitro-α-acetylamino-β-hydroxypropiophenone) test, or HPLC should be used for

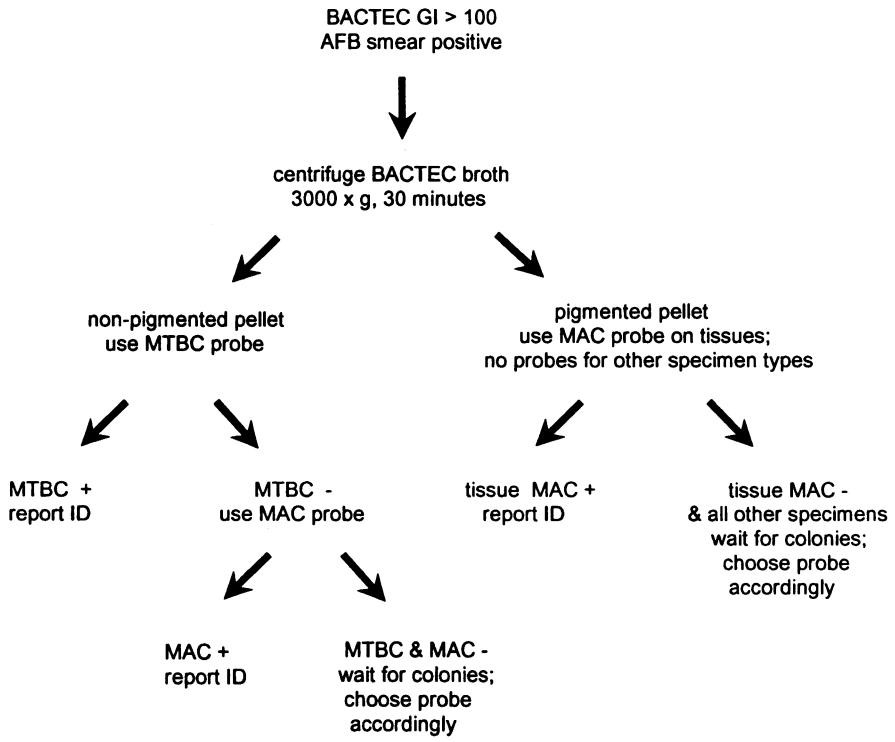

FIG. 1. Use of probes to identify mycobacteria from BACTEC broth cultures.

identification, and the result should be available within 21 days of receipt of the specimen. The mycobacterial culture and identification system selected, however, must provide accurate results.

In our laboratory, the BACTEC TB system is used to culture for mycobacteria; and for rapid identification during the period reviewed for the present study, growth from AFB-positive vials was tested with probes for MTBC, MAC, *M. kansasii*, and *M. gordonae*. Of 359 AFB-positive BACTEC vials, 75 of the 86 vials containing MTBC (87.2%) were identified after the initial screening of broth with probes. This percentage is much higher than the 47% reported by Evans et al. (6) but lower than the 100% reported by Thompson et al. (16) and Chapin-Robertson et al. (3). The fact that we tested many more MTBC-positive cultures than did Thompson et al. (16) or Chapin-Robertson et al. (3) may explain the difference between their results and ours. It is unclear why there is such a discrepancy between our MTBC probe results and those of Evans et al. (6).

Our results obtained with the MAC probe are very different from those that others have reported. In our experience, 99 (78.5%) of the 126 cultures containing MAC were initially positive by probe analysis. This percentage is much lower than the 90, 100, and 89% reported previously (3, 6, 16). Possible explanations for our low percentage include the GIs of false-negative vials and the centrifugation conditions used to prepare pellets for probe analysis.

Although a low GI certainly could be a factor, our analysis did not show a definite correlation between GI and a positive or a negative initial MAC probe result. For vials that were positive on initial testing, the GIs ranged from 18 to >999. The GIs of false-negative vials ranged from 108 to >999, 67% had GIs of >500, and 52% had GIs of >999. There was a similar lack of correlation between the GI and the initial probe result for the other species.

The centrifugation conditions that we used to prepare the pellets for probe analysis (3,000 × *g* for 15 min) differed from those used in the three previous studies (15,000 or 13,000 × *g* for 15 min or 10,000 × *g* for 5 to 7 min). The lower centrifugal force used in our protocol may have adversely affected the recovery and identification of organisms from broth cultures. In laboratories such as ours where a high-speed centrifuge is not currently available, increasing the centrifugation time may compensate for the lower centrifugal force. To address this possibility, we have recently doubled the centrifugation time in our protocol.

In our experience with the *M. gordonae* probe, 85.9% of isolates were detected upon initial screening of the BACTEC broth. This is lower than the 100% detected by Chapin-Robertson et al. (3); however, there were only six *M. gordonae* in that study, making comparison of the results difficult.

To date, data concerning the reliability of the *M. kansasii* AccuProbe have come from studies in which the probe was used for culture confirmation only. LeBrun et al. (12), who evaluated nine isolates of *M. kansasii*, reported probe sensitivities of 44%. Surprisingly, our detection rate when we used the probe for direct testing from a BACTEC TB vial was much higher (91.7%). However, the number of *M. kansasii* isolates in both studies (12 and 9) was small. In addition, the currently recommended incubation time for the selection step with the *M. kansasii* probe is 8 min, whereas it was 5 min in the study by LeBrun et al. (12). According to the manufacturer, this change was made to increase specificity, but it also may have an impact on sensitivity.

Given the current emphasis on cost containment in the health care industry, the selection of a rapid, cost-efficient protocol for the detection and identification of mycobacteria is desirable. Although the BACTEC TB system is more expensive than conventional media and requires more technologist

time, its increased sensitivity and rapid growth detection improves patient care, justifying the higher cost. Use of the AccuProbes with the BACTEC TB system provides the most rapid means of identification, but the probes can and should be used in a more cost-effective manner than the approach that we have been using in our laboratory. The protocol that we recently adopted is the following (illustrated in Fig. 1). Growth resulting in a nonpigmented pellet is tested first with the MTBC probe; if it is MTBC negative, the pellet is then tested with the MAC probe. Growth resulting in a pigmented pellet is tested with the MAC probe when the growth is from a tissue specimen, in which the presence of MAC is likely to represent disease. All other isolates are identified from solid media with probes or by biochemical tests.

Discontinuation of the routine use of the MAC probe on pigmented pellets is supported by our experience that few MAC isolates are pigmented and that the probe identified only 78.6% of MAC isolates directly from BACTEC vials. Discontinuation of the use of the *M. kansasii* probe is justified on the basis of the small number of isolates. Finally, discontinuation of the use of the *M. gordonae* probe is reasonable because this organism is almost always considered a contaminant and therefore does not require the rapid detection provided by the use of probes on BACTEC broth cultures.

In conclusion, use of the BACTEC TB-AccuProbe combination is a simple, relatively reliable method for the rapid identification of the mycobacteria most commonly encountered in the clinical laboratory. However, review of our 19-month experience suggests that the testing protocol may be improved by modifying the technique for preparing pellets for probe analysis by increasing either centrifugation speed or time and limiting the use of probes as outlined in Fig. 1 to rapidly detect *M. tuberculosis* while minimizing the cost to the laboratory and the patient.

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