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# Improved Detection Times for *Mycobacterium avium* Complex and *Mycobacterium tuberculosis* with the BACTEC Radiometric System

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**A total of 2,559 routine clinical specimens were cultured for mycobacteria by using BACTEC Middlebrook 7H12 medium (BACTEC), Lowenstein-Jensen slants (LJ), and Mycobactosel selective Middlebrook 7H11 slants (M7H11). Thirty-three isolates (1.3%) of *M. avium* complex and 82 isolates (3.2%) of *M. tuberculosis* were recovered. The BACTEC mean detection time of *M. avium* complex from 27 smear-negative specimens was earlier than that of conventional media for both decontaminated respiratory specimens (BACTEC, 12 days; LJ, 32 days; and M7H11, 38 days) and untreated tissue and fluid specimens (BACTEC, 8 days; LJ, 30 days; and M7H11, 31 days). The sensitivity for smear-negative *M. avium* complex with BACTEC (74%) was comparable to that with LJ (74%) and M7H11 (63%). The mean detection times of *M. tuberculosis* from 56 smear-positive respiratory specimens were 8 days for BACTEC, 16 days for LJ, and 17 days for M7H11, and sensitivities for the detection of positive cultures were 98% for BACTEC, 76% for LJ, and 79% for M7H11. The BACTEC mean detection time of *M. tuberculosis* in smear-negative specimens was better for tissues and fluids (14 days) than for respiratory specimens (24 days). BACTEC yielded substantially earlier detection of *M. avium* complex from all specimen types and of *M. tuberculosis* from smear-positive respiratory specimens. The rapid identification and susceptibility testing of *M. tuberculosis* in BACTEC agreed completely with conventional tests and provided a 3-week reduction in median time to final reports.**

The BACTEC radiometric blood culture system (Johnston Laboratories, Inc., Towson, Md.) has been adapted for the detection of mycobacteria by the use of Middlebrook 7H12 medium containing [$^{14}$C]palmitic acid (12). The initial evaluations from major referral centers have shown equivalent recovery rates and improved detection times (13, 15) for both *M. tuberculosis* and mycobacteria other than *M. tuberculosis* (MOTT bacilli). Confirmation of these findings (2, 6, 14, 18) has recently shown the benefit of the combined use of the radiometric system and conventional media.

*M. avium* complex has long been recognized as a predominant cause of mycobacterial infections due to MOTT bacilli in the southeastern regions of this country (3, 4), but its relative importance in all geographic regions was not apparent until national isolation rates were published. In a review of clinically significant mycobacterial isolates reported by state public health laboratories to the Centers for Disease Control in 1980, the pathogenic isolates included 65% *M. tuberculosis*, 21% *M. avium* complex, and less than 6% for each of the remaining nine species (7). A similar survey of 44 state laboratories in 1979 found that *M. avium* complex was the most frequently isolated of the potentially clinically significant MOTT bacilli in all nine geographic regions of this country (8). A recent report from Wisconsin found that the number of cases due to *M. avium* complex and to *M. tuberculosis* were equal (16). Another report from the Midwest found a greater proportion of *M. avium* complex isolates but did not distinguish between colonization and disease (20).

*M. avium* complex represents 24% of all mycobacteria and 60% of all MOTT bacilli isolated in the Harborview Medical Center laboratory. Over 80% of these *M. avium* complex isolates have come from patients with potential disease from this organism. With the advent of disseminated infections due to *M. avium* complex in patients with acquired immune deficiency syndrome (1, 5, 9, 11, 21, 22), there has been increased pressure to improve the laboratory report time for mycobacteriology specimens. With this in mind, we were particularly interested in evaluating the ability of the BACTEC system to detect *M. avium* complex as well as *M. tuberculosis*.

This study also included evaluation of inhibitory Mycobactosel Middlebrook 7H11 slants (M7H11), selected for use by this laboratory to reduce contamination rate.

## MATERIALS AND METHODS

Specimens evaluated in this study included all respiratory specimens, tissues, and body fluids excluding urines. Sterile tissue homogenates and body fluids with volumes of <10 ml were plated directly onto mycobacterial media. Body fluids with volumes of ≥10 ml were subjected to centrifugation for 30 min at 5,000 × *g*, and the sediment was inoculated onto media. All respiratory and other specimens likely to contain bacteria other than mycobacteria were decontaminated by the 2% NaOH–*N*-acetyl-L-cysteine method (19) before inoculation.

All specimens were inoculated onto the following mycobacterial media: one Middlebrook 7H12 vial (BACTEC) containing [$^{14}$C]palmitic acid (Johnston Laboratories); one Lowenstein-Jensen slant (LJ; PML Microbiologicals, Tualatin, Oreg.); and one M7H11, a selective medium containing cycloheximide (360 μg/ml), nalidixic acid (20 μg/ml), and lincomycin (2 μg/ml; PML). The latter two media are considered conventional media for the purposes of this study. The BACTEC 7H12 received 0.2 ml of specimens from sterile body sites or 0.1 ml of decontaminated specimen concentrate with 0.1 ml of the antimicrobial additive PACT (polymyxin B, 50 U/ml; amphotericin B, 5 μg/ml; carbeni-

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TABLE 1. *Mycobacterium* spp. recovered during 13-month study[a]

| Species | No. of isolates |
|---|---|
| *M. tuberculosis* | 82 |
| *M. avium* complex | 33 |
| *M. gordonae* | 8 |
| *M. kansasii* | 4 |
| *M. chelonae* | 3 |
| *M. fortuitum* | 2 |
| *M. marinum* | 2 |
| *M. terrae* | 1 |
| *M. scrofulaceum* | 1 |
| *M. flavescens* | 1 |

[a] A total of 137 isolates was recovered from 2,559 specimens.

cillin, 25 μg/ml; and trimethoprim, 2.5 μg/ml). Conventional media were inoculated with 0.1 ml of specimen.

Smears were prepared in duplicate from specimen sediments or tissue homogenates, and one smear of each set was stained for acid-fast bacilli (AFB) by the auramine method (19). Positive smears were confirmed by the Kinyoun acid-fast stain of the duplicate smears.

All media were incubated for 37°C in 5 to 10% $CO_2$. Conventional media were examined weekly for 8 weeks. BACTEC was flushed with 5 to 10% $CO_2$ prior to inoculation and read twice weekly on the BACTEC 460 instrument for the first 2 weeks and then once a week for an additional 4 weeks. Any vial with a growth index (GI; numerical value calculated within the instrument) of ≥10, indicating release of $^{14}CO_2$ from metabolism of the [$^{14}$C]palmitic acid, was read daily until a reading of ≥100 was obtained. As noted by the manufacturer, the sensitivity of AFB smears was inadequate to detect mycobacteria when the GI was less than 50 to 100. At a GI of ≥100, the vial contents were stained with auramine-O to confirm the presence of AFB, designated in Tables 3 and 4 as "BACTEC (confirmed AFB)." To prevent organisms from being washed off the slide, a drop of 20% phenolized serum was used in preparing the smear.

*M. tuberculosis* isolates were presumptively identified with BACTEC containing NAP (*p*-nitro-α-acetylamino-β-hydroxypropiophenone) and were confirmed by conventional biochemical testing (19). *M. avium* complex isolates were identified by conventional biochemical testing after growth in the NAP vial indicated the presence of MOTT bacilli.

Susceptibility testing was performed on *M. tuberculosis* by recommended inoculation procedures for the BACTEC system, which used final concentrations (in micrograms per milliliter) of 0.2 for isoniazid, 4.0 for streptomycin, 2.0 for rifampin, and 10.0 for ethambutol (10, 17). Susceptibility results were compared with results of the conventional agar plate method (19) performed by the Washington State Public Health Laboratories.

## RESULTS

From the 2,559 specimens evaluated between November 1982 and December 1983, 137 (5.3%) AFB isolates were recovered (Table 1). The time for detection and the rate of recovery were evaluated for 82 *M. tuberculosis* and 33 *M. avium* complex isolates, which represented 84% of all isolates.

The highest recovery rate in smear-positive *M. tuberculosis* specimens was found with the BACTEC system, which detected 98% of the isolates, compared with 76 and 79% on LJ and M7H11, respectively (Table 2). The yield from conventional media was 91%. The radiometric media had lower recovery rates for both smear-negative *M. tuberculosis* specimens (75%) and smear-negative *M. avium* complex specimens (74%) compared with rates of 92 and 82%, respectively, with the conventional media. However, the BACTEC recovery rate was comparable to that of either solid medium alone.

The BACTEC system provided more rapid detection of mycobacteria than did the conventional media. The improvement in detection time was not as great when based on times to observation of AFB rather than on a GI of ≥10 (Tables 3 and 4). The time between mean detection of GI of ≥10 and confirmation of AFB ranged from 0 to 10 days.

Comparison of the mean detection times on LJ and M7H11 showed that LJ provided improved detection times for four of the eight categories of specimens and was comparable to M7H11 for the remaining four categories (Tables 3 and 4). Therefore, the M7H11 results have been omitted from further comparisons of detection time results.

Smear-negative specimens containing *M. avium* complex showed the greatest improvement in mean detection times by BACTEC. The mean reduction in detection time by the BACTEC system with confirmed AFB was 15 days for respiratory specimens and 22 days for tissues and fluids.

Smear-negative specimens containing *M. tuberculosis* showed improved mean detection times in the BACTEC system when a GI of ≥10 was used. However, no improvement was noted for this specimen category when BACTEC mean detection times were based on confirmed AFB.

In smear-positive respiratory specimens, the reductions in mean times to detect confirmed AFB in BACTEC were 6 days for *M. tuberculosis* and 14 days for *M. avium* complex. Overall, the first detection of the presence of AFB was by BACTEC for 68 (83%) of the *M. tuberculosis* cultures and 23 (70%) of the *M. avium* complex cultures.

Susceptibility testing of *M. tuberculosis* in the BACTEC system was compared with the conventional agar plate method for 33 clinical isolates and was found to have 100% agreement. The tested isolates included 26 *M. tuberculosis*

TABLE 2. Recovery of *M. tuberculosis* and *M. avium* complex isolates on conventional media and the BACTEC system.

| Species | Direct smear result | No. of positive specimens | No. (%) of isolates recovered from: | | | |
|---|---|---|---|---|---|---|
| | | | LJ | M7H11 | Combined conventional LJ and M7H11 | BACTEC system |
| *M. tuberculosis* | Positive | 58 | 44 (76) | 46 (79) | 53 (91) | 57 (98) |
| | Negative | 24 | 19 (79) | 11 (46) | 22 (92) | 18 (75) |
| *M. avium* complex | Positive | 6 | 6 (100) | 6 (100) | 6 (100) | 6 (100) |
| | Negative | 27 | 20 (74) | 17 (63) | 22 (82) | 20 (74) |

isolates susceptible to isoniazid, streptomycin, rifampin, and ethambutol; 3 *M. tuberculosis* isolates resistant to isoniazid; 3 *M. tuberculosis* isolates resistant to streptomycin; and 1 *M. tuberculosis* isolate resistant to rifampin. The BACTEC susceptibility tests were completed in a mean of 6 days following inoculation of the drug vials.

The NAP differentiation test was used to distinguish *M. tuberculosis* from MOTT bacilli for 37 isolates and was found to agree completely with conventional biochemical testing. These isolates were recovered from either BACTEC or solid media and included the following isolates: 17 *M. tuberculosis*, 12 *M. avium* complex, 5 *M. gordonae*, 1 *M. chelonae*, 1 *M. fortuitum*, and 1 *M. kansasii*. The mean time from inoculation of the test vial to a presumptive *M. tuberculosis* or MOTT bacilli result was 5 days.

Figure 1 illustrates the time to final identification with susceptibility reports for *M. tuberculosis* for the two 8-month periods prior to and following the adoption of the BACTEC system. The median time to final reports was reduced by 19 days with the BACTEC system. Only 1 (6%) of 17 final reports was available in less than 44 days by conventional procedures compared with 12 (80%) of 15 completed in the same length of time with BACTEC.

Contamination rates for the three isolation media were evaluated for the first 9 months of the study. Of 893 respiratory specimens processed during this time, BACTEC with PACT antimicrobial supplement showed the lowest contamination rate (2.4%) compared with LJ (7.9%) and M7H11 (4.5%). The incidence of respiratory cultures discarded due to overgrowth by bacteria or fungi in all three media was 1.7%. The contamination rates for all specimen types were 1.6% for BACTEC, 5.5% for LJ, 3.1% for M7H11, and 1.1% for the three media combined.

The evaluation of BACTEC also included an analysis of those cultures with a GI of ≥10 that did not contain mycobacteria. The overall incidence of false-positives in BACTEC for all specimen types was 4.5%. Of these false-positives, 55% occurred in untreated specimens containing leukocytes that metabolized the palmitic acid substrate and were easily recognized by the drop in GI within 1 to 2 days of the initial positive reading. Another 22% were grossly contaminated and were confirmed by Gram stain at the time the GI was ≥10. The remaining 23% were smear negative both by Gram stain and auramine stain and required subculture to solid media and incubation for up to 5 days to confirm bacterial contamination.

TABLE 3. Detection times for *M. avium* complex

| Medium | Detection time (days) for *M. avium* complex smear | | | |
|---|---|---|---|---|
| | Positive | | Negative | |
| | Respiratory[a] (*n* = 4) | Tissues and fluids (*n* = 2) | Respiratory (*n* = 17) | Tissues and fluids (*n* = 10) |
| BACTEC (GI ≥ 10) | 3–7 (4) | 6 | 3–23 (12) | 7–9 (8) |
| BACTEC (confirmed AFB) | 3–7 (5) | 6 | 6–43 (17) | 7–9 (8) |
| LJ | 7–11 (9) | 12–32 (22) | 18–50 (32) | 11–47 (30) |
| M7H11 | 7–11 (9) | 12–44 (28) | 15–67 (38) | 11–47 (31) |

[a] Decontaminated respiratory specimens. Numbers in parentheses are mean number of days until detection.

TABLE 4. Detection times for *M. tuberculosis*

| Medium | Detection time (days) for *M. tuberculosis* smear | | | |
|---|---|---|---|---|
| | Positive | | Negative | |
| | Respiratory[a] (*n* = 56) | Tissues and fluids (*n* = 2) | Respiratory (*n* = 14) | Tissues and fluids (*n* = 10) |
| BACTEC (GI, ≥10) | 2–35 (8) | 7–23 (15) | 14–39 (24) | 9–29 (14) |
| BACTEC (confirmed AFB) | 3–43 (10) | 15–36 (25) | 20–48 (32) | 11–45 (24) |
| LJ | 9–27 (16) | 16 | 21–42 (31) | 15–58 (29) |
| M7H11 | 10–44 (17) | 22–42 (32) | 28–67 (45) | 19–34 (29) |

[a] Decontaminated respiratory specimens. Numbers in parentheses are mean number of days until detection.

## DISCUSSION

This study compared the BACTEC system with LJ and M7H11 for the detection of *M. tuberculosis* and *M. avium* complex in both smear-positive and smear-negative specimens. Results from decontaminated respiratory specimens and untreated tissue and fluid specimens were separated to evaluate the effects of decontamination on recovery rate and detection time.

The results from smear-positive respiratory specimens containing *M. tuberculosis* were essentially the same as those described in the large multicenter BACTEC study reported by Roberts et al. (15). The sensitivity of the BACTEC system for detecting *M. tuberculosis* in our specimens was 98%, which was comparable to the 94.6% reported by Roberts et al. The BACTEC mean detection time of *M. tuberculosis* in smear-positive respiratory specimens in our study (8 days) was also very similar to the 8.3-day detection time in the study by Roberts et al.

*M. avium* complex was recovered from all media inoculated with the 6 smear-positive specimens containing *M. avium* complex, but of the 27 smear-negative specimens containing this organism, only 20 (74%) grew in BACTEC, and 22 (82%) grew on conventional media. These results, which presumably reflect the low numbers of organisms present in smear-negative specimens, are similar to the results from the Mayo Clinic study of 37 smear-negative specimens containing *M. avium* complex in which BACTEC recovered 32 (86%) isolates, and conventional media yielded 31 (84%) isolates.

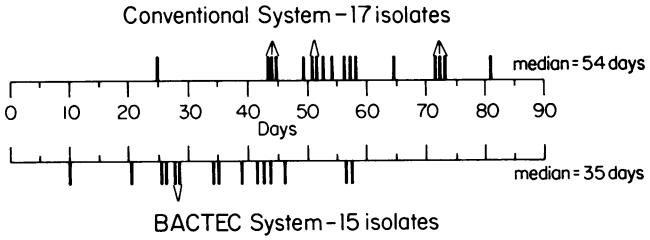


FIG. 1. Days to final reports of *M. tuberculosis* identification with susceptibility results. Data for the conventional system were collected from April through November 1982, prior to adoption of the BACTEC system. Data for the BACTEC system were collected from January through August 1984, after susceptibility test drugs became available.

The mean detection times of *M. tuberculosis* from smear-negative specimens in BACTEC differed for untreated specimens (14 days) and decontaminated specimens (24 days), but detection times on LJ were comparable for both types of specimens (29 and 31 days, respectively). If positive results were calculated according to the time at which AFB were observed in the vials (confirmed AFB), BACTEC provided no advantage in mean detection time of *M. tuberculosis* from smear-negative decontaminated specimens and a 5-day advantage over conventional media for untreated specimens. The two earlier BACTEC studies that reported recovery in BACTEC of *M. tuberculosis* from smear-negative specimens combined the data from both decontaminated and untreated specimens and found mean detection times of 13.7 days (13) and 20 days (14), results which were similar to the results in this study.

BACTEC provided a substantial improvement in time to detection of *M. avium* complex from both decontaminated and untreated smear-negative specimens. Mean detection time in BACTEC of confirmed-positives from untreated tissues and fluids was 8 days, compared with 30 days on LJ. In the report by Morgan et al., which combined the data from both decontaminated and untreated specimens, BACTEC also detected *M. avium* complex within 8 days, but the time to detection on their conventional media was 20 days (13). The ratio of total inoculum volumes in conventional media versus in BACTEC was 15:1 in the previous study (13), compared with 2:1 for decontaminated specimens and 1:1 for untreated specimens in our study. The long detection times on conventional media may also be attributed to poor visibility of transparent *M. avium* complex colonies on slants compared with visibility on plate media.

The detrimental effect on detection time of decontamination was evident for both smear-negative *M. tuberculosis* and *M. avium* complex cultures. The observed delays in detection time can be attributed to the combined effects of low numbers of organisms in the original specimens, damage incurred during the decontamination procedure, and inhibitory effects of the antimicrobial agents in PACT-supplemented BACTEC or in M7H11. These results are consistent with a recent study of smear-positive specimens from treated patients in which decontamination of specimens caused marked delays in mycobacterial growth when it was used in combination with selective solid media or BACTEC broth containing the PACT supplement (2).

The difference in inoculum volume between decontaminated and untreated specimens in BACTEC may also have an effect on relative detection times and recovery rates. BACTEC received twice the inoculum volume for untreated specimens compared with decontaminated specimens and compared with each solid medium.

The incidence of contamination for 58 smear-positive *M. tuberculosis* specimens was 6 specimens on LJ and 0 specimens in BACTEC. Presumably, the 76% recovery from LJ would have been raised to 86% if these slants had not been overgrown with contaminants.

The poor performance of the inhibitory M7H11 in detection time and recovery rate confirmed previous reports showing reduced sensitivity with combined use of decontamination and selective media (2). Additionally, the low contamination rate in BACTEC (2.4%) compared with M7H11 (4.5%) eliminated the basis for using this medium. We have recently replaced the inhibitory M7H11 slants with nonselective Middlebrook 7H11 slants to improve recovery and reduce detection time on conventional media. Although we would predict a greater improvement in detection time with a Middlebrook 7H11 plate medium, especially for the *M. avium* complex, incubator space limitations due to the adoption of the BACTEC system have precluded its use.

In our experience, the incidence in BACTEC of a false-positive GI of $\geq$10 (4.5%) was similar to that for true-positives (5.4%). Because 23% of the false-positives could not be resolved before the results of subcultures were known, we are not reporting positive cultures based on a GI of $\geq$10 until smear confirmation of AFB in the vials is obtained. Although this policy occasionally results in withholding important information for a few days, it provides the greater advantage of generating a high level of physician confidence in the laboratory results and frequently results in prompt changes in patient management when appropriate.

The rapid and reliable identification and susceptibility tests for *M. tuberculosis* in BACTEC provided a major improvement in the response of the laboratory to the clinicians. The time to detection of *M. avium* complex was markedly improved by the BACTEC system. Subculture and conventional biochemical testing were still necessary for identification, but for some acquired immune deficiency syndrome patients from whom isolates were taken from sterile sites, the treatment was initiated prior to final identification. When used in conjunction with solid media, the BACTEC system provided the most rapid recovery of mycobacteria while not sacrificing the valuable role that colonial morphology plays in the presumptive identification of MOTT bacilli.

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