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Multicenter Evaluation of the BACTEC MGIT 960 System for Recovery of Mycobacteria

BRUCE A. HANNA, 1* ADELEH EBRAHIMZADEH, 2 L. BRUCE ELLIOTT, 3 MARGIE A. MORGAN, 4 SUSAN M. NOVAK, 5 SABINE RUSCH-GERDES, 6 MILLIE ACIO, 4 DENISE F. DUNBAR, 3 T. MICHELE HOLMES, 3 CHARLES H. REXER, 1 CHIMINYAN SAVTHYAKUMAR, 2 AND ANN M. VANNIER 5

New York University School of Medicine, Bellevue Hospital, 1 and The City of New York Department of Health, 1 New York, New York; Texas Department of Health, Austin, Texas; 2 Cedars-Sinai Medical Center, Los Angeles, 5 and Kaiser Permanente, North Hollywood, 5 California; and Forschungszentrum Borstel, Borstel, Germany 6

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We evaluated the BACTEC MGIT 960 system, which is a fully automated, noninvasive system for the growth and detection of mycobacteria with a capacity to incubate and continuously monitor 960 7-ml culture tubes. We studied 3,330 specimens, 2,210 respiratory and 1,120 nonrespiratory specimens, collected from 2,346 patients treated at six sites. Processed specimens were inoculated into the BACTEC MGIT 960 and BACTEC 460 TB systems, as well as onto Lowenstein-Jensen slants and Middlebrook 7H11/7H11 selective plates. From all culture systems, a total of 362 isolates of mycobacteria were recovered; these were recovered from 353 specimens collected from 247 patients. The greatest number of isolates of mycobacteria (289, or 80% of the 362 isolates) was recovered with the BACTEC MGIT 960, followed by the BACTEC 460 TB (271, or 75%) and solid media (250, or 69%). From all culture systems a total of 362 isolates of Mycobacterium tuberculosis complex were recovered. The greatest number of isolates of M. tuberculosis complex was recovered when liquid medium was combined with conventional solid media; the number recovered with BACTEC 460 TB plus solid media was 128 (97%), that recovered with BACTEC MGIT 960 plus solid media was 121 (92%), that recovered with BACTEC 460 TB was 119 (90%) and that recovered with all solid media combined was 105 (79%). The recovery with BACTEC MGIT 960 alone was 102 (77%). The mean times to detection (TTD) for M. tuberculosis complex were 14.4 days for BACTEC MGIT, 15.2 days for BACTEC 460 TB, and 24.1 days for solid media. The numbers of isolates of Mycobacterium avium complex (MAC) recovered were 172 (100%) for all systems, 147 (85%) for BACTEC MGIT 960, 123 (72%) for BACTEC 460 TB, and 106 (62%) for all solid media combined. The TTD for MAC in each system were 10.0 days for BACTEC MGIT 960, 10.4 days for BACTEC 460 TB, and 25.9 days for solid media. Breakthrough contamination rates (percentages of isolates) for each of the systems were 8.1% for BACTEC MGIT 960, 4.9% for BACTEC 460 TB, and 21.1% for all solid media combined.

The increasing incidence of tuberculosis and other mycobacterial diseases has made it essential for laboratories to quickly detect and identify mycobacteria from human clinical material. When conventional culture media are used, as many as several weeks of incubation and substantial technical labor may be necessary for the recovery of organisms. Since it was first introduced, the BACTEC MGIT 960 system (Becton Dickinson Microbiology Systems, Sparks, Md.) has been the benchmark for rapid detection of Mycobacterium tuberculosis complex (8, 9). In recent years, however, a number of new systems which provide similar times to detection, with fully automated instruments or without the need for any instrumentation, have been developed. The BACTEC MGIT 960 system is a fully automated, high capacity, nonradiometric, noninvasive system which requires neither needles nor other sharp implements to simultaneously incubate and monitor 960 7-ml culture tubes. To monitor microbial growth, the BACTEC MGIT 960 uses the same oxygen-quenching fluorescent sensor technology as both the manual Mycobacteria Growth Indicator Tube (BBL MGIT) and the BACTEC 9000MB system, in conjunction with unique on-board algorithms to determine the positivity of the culture tubes. This multicenter study evaluated the performance of the BACTEC MGIT 960 as compared to that of the BACTEC 460 TB system as well as to those of the conventional Lowenstein-Jensen (LJ) and Middlebrook 7H11 solid media for the recovery of mycobacteria from human clinical specimens.

MATERIALS AND METHODS

Test sites. Diverse populations of patients treated at six test sites participated in the study. Three of the sites were health department laboratories (one metropolitan facility, one state facility, and one European regional reference center). The other three sites were a university-based tertiary care medical center, a private medical center, and a regional reference center.

Specimens. From among specimens submitted to the mycobacteriology laboratories at each of the six study sites, 3,330 specimens collected from a total of 2,346 patients were selected at random. Specimen distribution included 2,210 respiratory and 1,120 nonrespiratory samples. All specimens were digested and decontaminated according to standard Centers for Disease Control methods (6). Specimens were processed by the sodium hydroxide and N-acetyl-L-cysteine (NaOH/NALC) method, with final concentrations of 1% for NaOH and 0.25% for NALC at five sites (sites A through E) and 2% for NaOH and 0.25% for NALC at one site (site F).

AFB smears. Using the sediment, smears were prepared from all specimens other than urine and were examined for acid-fast bacteria (AFB). All AFB smears were either stained with auramine and examined with a fluorescent microscope (five sites) or stained by the Kinyoun method and examined with a light microscope (one site).

Culture medium inoculation, incubation, and test duration. The remaining sediment was suspended in 1 to 2 ml of sterile phosphate-buffered saline (pH 6.8) and vortexed for 15 s. This suspension was then used for culture medium inoculation. The order of inoculation for each of the broth media systems was varied. After all liquid media were inoculated, 0.1 to 0.25 ml of each processed specimen was also inoculated onto the surface of an LJ slant and onto each half of a
TABLE 1. Specimen types and recovery of mycobacteria

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>No. of specimens</th>
<th>No. of positive specimens (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td>2,210</td>
<td>321 (14.5)</td>
</tr>
<tr>
<td>Body fluids</td>
<td>464</td>
<td>4 (0.9)</td>
</tr>
<tr>
<td>Tissue</td>
<td>595</td>
<td>23 (3.8)</td>
</tr>
<tr>
<td>Stool</td>
<td>61</td>
<td>3 (4.9)</td>
</tr>
<tr>
<td>Urine</td>
<td>167</td>
<td>0</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>18</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>Other*</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>3,330</td>
<td>353 (10.6)</td>
</tr>
</tbody>
</table>

* Specimen source not specified.

7H11/7H11 selective biplate. All culture media were incubated at 37°C, while solid media were incubated in an atmosphere of 5% to 10% CO₂. All liquid medium cultures were tested either until found to be positive or for 42 days. Solid media were tested for 56 days.

Culture systems. (i) BACTEC MGIT 960. The BACTEC MGIT 960 culture tube contains 7 ml of Middlebrook 7H9 broth base, to which was added an enrichment supplement containing oleic acid, albumin, dextrose, and catalase (BBL MGIT OADC) and an antibiotic mixture of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (BBL MGIT PANTA). After inoculation of each tube with 0.5 ml of the processed specimen, the tubes were incubated at 37°C in the BACTEC MGIT 960 instrument and were monitored automatically every 60 min for increase of fluorescence. A series of algorithms included in the instrument is used to determine presumptive positivity and to alert the operator to the presence and locations of the positive tubes by means of indicator lights positioned on the front of the instrument, tube status displayed on the video display screen, and LEDs positioned at the appropriate tube station. Any sample which was identified as positive was removed from the instrument, and a smear was prepared and examined for AFB. Time to detection of mycobacteria was based on the earliest date of detection of colonies on any of the solid media, ultimately confirmed by positive AFB smear.

(ii) BACTEC 460 TB. BACTEC 12B culture vials were prepared according to the manufacturer’s instructions and inoculated with 0.5 ml of the processed specimen. Vials were monitored two to three times weekly for the first 2 to 3 weeks and then once weekly thereafter through the sixth week with the BACTEC 460 TB System. Any vial which was identified as having a growth index (GI) of 10 or greater was considered presumptively positive and was tested daily until a GI of ≥50 was observed, whereupon an AFB smear was prepared and examined. For the study, the times to detection (TTD) were based on the first date that a GI of ≥50 was found to correlate with AFB smear positivity.

(iii) Solid media. All specimens were inoculated onto conventional solid media, which included one LJ slant (BBL) and one 7H11/7H11 selective agar biplate (BBL). For the purpose of data analysis, the LJ slant and the Middlebrook biplate were regarded as a single solid-medium system. An individual medium was considered positive upon appearance of colonies on the surface, and the TTD was based on the earliest date of detection of colonies on any of the solid media, ultimately confirmed by positive AFB smear.

Culture protocol. For all systems, when the first culture medium of a cohort was identified as positive, an AFB smear (Kinyoun or Ziel-Neelsen) was prepared the same day. If this smear was prepared from a broth medium and it was positive for AFB, the broth was subcultured. If, however, this smear was prepared from a solid medium and it was positive for AFB, no further subculture was made. The AFB recovered from solid or liquid media were then identified by the AccuProbe (Gen-Probe, Inc.) culture identification test, high-pressure liquid chromatography, and/or conventional biochemical tests. If, on the other hand, the AFB smear from any flagged positive medium was negative, a gram-stained smear was prepared and examined and the result was noted. If a presumptively positive broth medium was positive for a bacterium that was not AFB but was negative for AFB, the medium was considered positive only for breakthrough contaminants and was not subjected to further testing. At the end of the instrument protocol period, a terminal AFB smear and subculture to Middlebrook media were prepared from all instrument-negative liquid media for which any cohort medium was positive for mycobacteria.

RESULTS

We compared 3,330 specimens collected from 2,346 patients treated at the six sites. By all culture systems, a total of 362 isolates of mycobacteria were recovered from 353 specimens, which had been collected from 247 patients. The majority of specimens tested (2,210 specimens [66%]) were from the respiratory tract. Overall, 353 (10.6%) of the specimens tested were positive for mycobacteria (Table 1). Among the respiratory specimens, 321 (14.5%) were positive. Mycobacteria were also recovered from 2 (11.1%) of 18 bone marrow specimens, 23 (5.8%) of 395 tissue specimens, 3 (4.9%) of 61 stool specimens, and 4 (0.9%) of 464 body fluid specimens. Although 167 urine samples were tested, none were positive by any medium used in the study.

The numbers of isolates of M. tuberculosis complex, Mycobacterium avium complex (MAC), and mycobacteria other than M. tuberculosis (MOTT, not inclusive of MAC) recovered in each of the culture systems as well as the combined result of pairing each of the liquid media with solid media are listed in Table 2. Of the 362 isolates recovered during the study, there were 172 (47.5%) isolates of MAC, 132 (36.5%) isolates of M. tuberculosis complex, and 58 (16%) isolates of MOTT recovered by all systems and media. As a single system, the BACTEC MGIT 960 detected the greatest number of mycobacteria, 289 isolates (80%), followed by the BACTEC 460 TB System, with 271 isolates (75%), and the solid media, with 250 isolates (69%). When each of the broth medium systems was paired with the solid media and the combined recovery rates were compared, the same trends were noted. The BACTEC MGIT 960 plus solid media detected 335 mycobacteria (93%), as contrasted with 308 mycobacteria (85%) recovered in the BACTEC 460 TB system plus solid media.

As shown in Table 2, of the 132 isolates of M. tuberculosis complex recovered during the study, 119 (90%) were detected with the BACTEC 460 TB System, as contrasted with 105 (79%) detected with solid media and 102 (77%) detected with the BACTEC MGIT 960. When recovery on the combined solid media was paired with that on the broth systems, the BACTEC 460 TB System plus solid media detected 128 (97%) of the M. tuberculosis isolates as contrasted with 121 (92%) detected with the BACTEC MGIT 960 plus solid media.

Of the 172 MAC isolates, more were recovered in the BACTEC MGIT 960 (147 isolates, or 86%) than in the BACTEC 460 TB system (123 isolates, or 72%) and with the combined solid media (106 isolates, or 62%). When the combined solid media were paired with the broth systems, the recovery of MAC was also greater with BACTEC MGIT 960.

TABLE 2. Distribution of isolates recovered in each culture system and in combinations of solid and liquid systems

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total, all media</th>
<th>960 + solid media</th>
<th>460 TB + solid media</th>
<th>Total, 960</th>
<th>Total, 460 TB</th>
<th>Total, solid media</th>
<th>960 only</th>
<th>460 only</th>
<th>Solid media only</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. tuberculosis complex</td>
<td>132 (100)</td>
<td>121 (92)</td>
<td>128 (97)</td>
<td>102 (77)</td>
<td>119 (90)</td>
<td>105 (79)</td>
<td>4 (3)</td>
<td>11 (8)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>MAC</td>
<td>172 (100)</td>
<td>160 (93)</td>
<td>136 (79)</td>
<td>147 (86)</td>
<td>123 (72)</td>
<td>106 (62)</td>
<td>36 (21)</td>
<td>12 (5)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>MOTT</td>
<td>58 (100)</td>
<td>54 (93)</td>
<td>44 (76)</td>
<td>40 (69)</td>
<td>29 (50)</td>
<td>39 (67)</td>
<td>14 (24)</td>
<td>4 (7)</td>
<td>10 (17)</td>
</tr>
<tr>
<td>All</td>
<td>362 (100)</td>
<td>335 (93)</td>
<td>308 (85)</td>
<td>289 (80)</td>
<td>271 (75)</td>
<td>250 (69)</td>
<td>54 (15)</td>
<td>27 (7)</td>
<td>16 (4)</td>
</tr>
</tbody>
</table>

* 960, BACTEC MGIT 960 system; 460 TB, BACTEC 460 TB system.
plus solid media (160 isolates, or 93%) than with the BACTEC 460 TB system plus solid media (136 isolates, or 79%).

As a single system, the BACTEC MGIT 960 detected 40 (69%) of the 58 isolates of MOTT, whereas 39 (67%) of these isolates were detected with solid media and only 29 (50%) isolates were detected in the BACTEC 460 TB system. When the broth systems were paired with the solid media, the BACTEC MGIT 960 and solid media recovered 54 isolates (93%), whereas 44 (76%) isolates were found with the BACTEC 460 TB system and solid media.

As may be expected, there were several instances where one culture system was scored as contaminated while other media in the cohort were observed to be positive for AFB. In the present study, this occurred more often with the BACTEC MGIT 960 than with the other systems. There were 20 specimens for which mycobacteria were recovered from other cohort media while only breakthrough contaminants were detected in the BACTEC MGIT 960 vial. In comparison, there were only eight specimens for which mycobacteria were recovered in other cohort media while only breakthrough contaminants were detected in the BACTEC 460 TB System.

In Table 3, the distribution of isolates recovered in culture system and combinations with contaminated cohorts removed from analysis is shown. The data includes the total number of isolates recovered with both systems, the BACTEC MGIT 960 and BACTEC 460 TB systems for the paired isolates, and the BACTEC MGIT 960 and BACTEC 460 TB systems for the nonpaired isolates. The table also includes the total for isolates recovered with either system and the total for isolates recovered with both systems.

In Table 4, the TTD (time to detection) for M. tuberculosis complex and MAC in the BACTEC MGIT 960 and BACTEC 460 TB systems for the paired and nonpaired isolates are shown. The TTD is defined as the detection of any instrument-positive result for the paired and nonpaired isolates. The table includes the TTD for the paired and nonpaired isolates, grouped by whether the AFB smear result was negative or positive. Among both the paired and the nonpaired AFB smear-positive specimens, there was very little difference between the TTD for the BACTEC 460 TB system (10.8 days for nonpaired specimens and 10.3 days for paired specimens) and the BACTEC MGIT 960 (10.6 days for nonpaired specimens and 10.8 days for paired specimens). Notably, as can be seen in this table, the increased number of M. tuberculosis complex isolates recovered in the BACTEC 460 TB system was due almost exclusively to recovery from smear-negative samples. This may be a result of random inoculum distribution, particularly when small numbers of organisms are present in the original specimen.

Table 6 shows the breakthrough contamination rates for the BACTEC MGIT 960 and BACTEC 460 TB systems for specimens collected at all sites in the study. Breakthrough contamination was defined as the detection of any instrument-positive result for the paired and nonpaired isolates.

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**TABLE 3. Distribution of isolates recovered in culture system and combinations with contaminated cohorts removed from analysis**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total no. of isolates, all media</th>
<th>Total no. (%) of isolates recovered witha:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>960 + solid media</td>
<td>460 TB + solid media</td>
</tr>
<tr>
<td>M. tuberculosis complex</td>
<td>118</td>
<td>111 (94)</td>
</tr>
<tr>
<td>MAC</td>
<td>160</td>
<td>153 (96)</td>
</tr>
<tr>
<td>MOTT</td>
<td>53</td>
<td>49 (92)</td>
</tr>
<tr>
<td>All</td>
<td>331</td>
<td>313 (95)</td>
</tr>
</tbody>
</table>

a 960, BACTEC MGIT 960 system; 460 TB, BACTEC 460 TB system.

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**TABLE 4. TTD of M. tuberculosis, MAC, and all mycobacteria in BACTEC MGIT 960 and BACTEC 460 TB systems**

<table>
<thead>
<tr>
<th>Culture system</th>
<th>M. tuberculosis complex</th>
<th>MAC</th>
<th>All mycobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonpairedb</td>
<td>Pairedb</td>
<td>Nonpairedc</td>
</tr>
<tr>
<td>BACTEC 960</td>
<td>14.4 (102)</td>
<td>14.4 (92)</td>
<td>10.0 (147)</td>
</tr>
<tr>
<td>BACTEC 460</td>
<td>15.2 (119)</td>
<td>13.2 (92)</td>
<td>11.4 (130)</td>
</tr>
</tbody>
</table>

a Total for isolates recovered with either BACTEC MGIT 960 or BACTEC 460 TB system.
b Total for isolates recovered with both systems.
c P = 0.055, not significant.
d P = 0.001.
tube that was AFB smear negative and subculture negative yet gram-stain smear positive for other (non-acid-fast) microorganisms. The contamination rate (percentage of isolates) noted with the BACTEC MGIT 960 was 8.1% (range, 1.8 to 14.6%), while that with the BACTEC 460 TB system was 4.9% overall (range, 0.9 to 9.2%). As shown in Table 6, at two of the trial sites, the contamination rates for the two systems were similar, while at the other four sites the contamination rate noted for the BACTEC MGIT 960 was higher than that noted for the BACTEC 460 TB.

The false-positive rate for the BACTEC MGIT 960 was 0.8% and was calculated as the number of BACTEC MGIT 960 vials which were instrument positive but were found to be smear negative and subculture negative for mycobacteria or other bacteria. During the study a total of 351 randomly selected BACTEC MGIT 960 instrument-negative vials (at the end of the 42-day protocol) were examined by AFB smear and subcultured to solid media. No false-negative samples from these random terminal subcultures of instrument-negative tubes were detected during the study.

**DISCUSSION**

Following the recent resurgence of tuberculosis in the United States, the rapid diagnosis of patients with active disease has become a focus of much interest (1, 11). In addition, MAC has become the systemic bacterial pathogen most commonly recovered from patients with AIDS and is an increasingly recognized pulmonary pathogen in nonimmunocompromised individuals as well (3). While amplified nucleic acid hybridization probe assays for M. tuberculosis complex provide an important adjunct to the diagnostic armamentarium, they have yet to displace traditional culture (2, 4). Among specimens collected at all sites, 48% of the isolates were recovered in the BACTEC 460 TB system and with solid media. However, the false-positive rate for the BACTEC MGIT 960 was 0.8% and was calculated as the number of BACTEC MGIT 960 vials which were instrument positive but were found to be smear negative and subculture negative for mycobacteria or other bacteria. During the study a total of 351 randomly selected BACTEC MGIT 960 instrument-negative vials (at the end of the 42-day protocol) were examined by AFB smear and subcultured to solid media. No false-negative samples from these random terminal subcultures of instrument-negative tubes were detected during the study.

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**TABLE 6. Breakthrough contamination rates**

<table>
<thead>
<tr>
<th>System</th>
<th>All sites (n = 3,330)</th>
<th>Site A (n = 558)</th>
<th>Site B (n = 494)</th>
<th>Site C (n = 679)</th>
<th>Site D (n = 628)</th>
<th>Site E (n = 323)</th>
<th>Site F (n = 648)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BACTEC MGIT 960</td>
<td>8.1</td>
<td>9.3</td>
<td>5.4</td>
<td>10.0</td>
<td>14.6</td>
<td>1.8</td>
<td>4.0</td>
</tr>
<tr>
<td>BACTEC 460 TB</td>
<td>4.9</td>
<td>5.0</td>
<td>0.6</td>
<td>9.2</td>
<td>7.1</td>
<td>0.9</td>
<td>3.7</td>
</tr>
<tr>
<td>Combined solid media</td>
<td>21.1</td>
<td>27.4</td>
<td>0.8</td>
<td>13.2</td>
<td>31.0</td>
<td>5.2</td>
<td>37.8</td>
</tr>
</tbody>
</table>
960 more closely approximated that of the BACTEC 460 TB system. On the other hand, when specimens from which MAC was recovered from a cohort vial but the other was contaminated were removed from the analysis, the superiority of the BACTEC MGIT 960, either with or without solid media, remained significant.

In addition to overall recovery, the TTD is an important performance characteristic of mycobacterial detection systems. When all isolates recovered were included in the analysis, the TTD of M. tuberculosis complex with the BACTEC MGIT 960 (14.4 days) was faster than that with the BACTEC 460 TB system (15.2 days). When only the 92 paired isolates (recovered in both broth systems) were included in the analysis, however, the TTD with the BACTEC 460 TB system (13.2 days) was faster than that with the BACTEC MGIT 960 (14.4 days). The reason for this is apparent in Table 5, where all M. tuberculosis complex isolates recovered in each system are separated according to smear result. The higher recovery rate of these isolates in the BACTEC 460 TB system was almost exclusively due to recovery from smear-negative specimens, which may be expected to have fewer bacilli in the originating specimen and, concomitantly, a longer mean TTD. As seen in Table 4, among the total AFB-containing samples, the TTD recorded for the two BACTEC systems were essentially the same, regardless of AFB smear result.

As a whole, the rate of breakthrough contamination was found to be higher with the BACTEC MGIT 960 (8.1%) than with the BACTEC 460 TB system (4.9%). There was, however, a large variation in the contamination rate among the test sites for all the media tested. These wide variations may reflect the dissimilar conditions of specimen quality and transport time and conditions among the sites. Notably, at two of the six test sites there was little difference in the rate of contamination between the two systems, while at the other four sites, contamination rates were clearly higher with the BACTEC MGIT 960 than the BACTEC 460 TB system. This is similar to the experiences reported when the ESP (13), MB/BacT System (10), BACTEC 9000MB (14), and BBL MGIT (15) were compared to the BACTEC 460TB system. These higher rates of breakthrough contamination are likely a result of the fact that the media in the newer systems are more enriched than the BACTEC 460 TB media. The BACTEC 12B medium relies on the metabolic utilization of radiolabeled palmitic acid for the detection of $^{14}$C-labeled CO$_2$, signaling the presence of growing mycobacteria. Other than the radiolabeled substrate, the 12B medium is very low in nutrient content and is not an optimal growth environment for most bacteria, which do not utilize the $^{14}$C substrate and thus are not detected.

The amounts of labor and user interaction required for efficient operation are a substantial consideration in the selection of any microbiology system. The BACTEC MGIT 960 system has very good performance characteristics, is easy to use, and readily fits into the customary mycobacteriology laboratory work flow. In large-volume laboratories, the 960-culture tube capacity provides a decided advantage over the continuously monitored instruments of lesser capacity. When a 42-day protocol is used in a laboratory with a 10% positivity rate, a single BACTEC MGIT 960 system could reasonably be expected to accommodate at least 8,000 specimens per year.

In summary, the newly introduced BACTEC MGIT 960 system is a dependable, high-capacity, compact, fully automated continuous monitoring instrument for the recovery of mycobacteria from human clinical samples. When used in combination with solid media, the BACTEC MGIT 960 system shows performance comparable to that of the BACTEC 460 TB system for the detection of M. tuberculosis complex, while providing greater recovery of MAC and MOTT.

ACKNOWLEDGMENT

This study was supported by Becton Dickinson Microbiology Systems.

REFERENCES