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# Comparison of Recoveries of *Mycobacterium tuberculosis* Using the Automated BACTEC MGIT 960 System, the BACTEC 460 TB System, and Löwenstein-Jensen Medium

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**Using two different liquid media and one conventional solid medium, a total of 57 mycobacterial isolates (*Mycobacterium tuberculosis*, $n = 55$; nontuberculous mycobacteria, $n = 2$) were recovered from 377 clinical specimens. The rates of recovery of *M. tuberculosis* were 96.4% with the BACTEC MGIT 960 liquid medium, 92.7% with BACTEC 12B liquid medium, and 81.8% with the Löwenstein-Jensen (LJ) medium. The mean time to detection of *M. tuberculosis* in smear-positive specimens was 12.6 days for BACTEC MGIT 960 medium, 13.8 days for BACTEC 12B medium, and 20.1 days for LJ medium, and in smear-negative specimens it was 15.8 days for BACTEC MGIT 960 medium, 17.7 days for BACTEC 12B medium, and 42.2 days for LJ medium. The rates of contamination were 3.7, 2.9, and 1.2% for the BACTEC MGIT 960, BACTEC 12B, and LJ media, respectively. In conclusion, the nonradiometric, fully automated 7-ml BACTEC MGIT 960 system can be considered a viable alternative to the semiautomated, radiometric BACTEC 460 TB system.**

The use of the radiometric BACTEC 460 TB broth-based system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) considerably improves the recovery of and decreases the time required to detect mycobacteria; however, this procedure is still labor-intensive and requires attention to special safety and regulatory issues regarding radioisotopes (7). Previous reports have demonstrated that the 4-ml Mycobacteria Growth Indicator Tube (MGIT; BBL Becton Dickinson Microbiology Systems, Cockeysville, Md.) and MB Redox (Biotest AG, Dreieich, Germany) systems are suitable nonradiometric alternatives to BACTEC 460 TB (4, 5, 8). However, these methods still require manual processing and are best suited for laboratories which cannot afford or, due to the low number of processed specimens, do not need instrumentation. Automation of the cultivation process is high on the list of priorities for laboratories dealing with large specimen loads. Although the recently developed MB/BacT (Organon Teknika, Turnhout, Belgium) and ESP II (Difco Laboratories, Detroit, Mich.) culture systems provide a fully automated, walk-away cultivation process, the capacity of these instruments is rather low (MB/BacT, 240 vials per instrument; ESP II, 384 vials per instrument) (1, 6, 9, 11). Therefore, several units are required, which might be expensive even for laboratories in high-income countries. The BACTEC MGIT 960 system is a high-capacity, fully automated, continuous-monitoring instrument that can test up to 960 7-ml MGIT vials for the presence of mycobacteria using nonradiometric fluorescence technology (2, 10). The culture vials contain a fluorescent sensor that responds to the concentration of oxygen in the culture medium. The instrument's photodetectors measure the fluorescence in each vial every 60 min. The level of fluorescence corresponds to the amount of oxygen consumed by the organisms in the inoculated specimens, and this, in turn, is proportional to the number of bacteria present. When a certain level of fluorescence is reached, the instrument indicates that the vial is positive. The purpose of this study was to evaluate the fully automated 7-ml BACTEC MGIT 960 system for the detection of mycobacteria in clinical specimens and compare the results with those of the reference BACTEC 460 TB system and of Löwenstein-Jensen (LJ) solid medium in terms of recovery rate, mean time to detection, and contamination rate.

The Department of Respiratory Medicine of Semmelweis Medical School is a university-based tertiary care medical center with its own laboratory facility. A total of 377 consecutive clinical specimens (288 sputum, 51 bronchoalveolar lavage or bronchial mucus aspirate, 32 gastric juice, and 6 pleural effusion) from 243 patients were processed between 29 March 1999 and 31 May 1999. All patients were human immunodeficiency virus negative. All clinical specimens were digested and decontaminated by the *N*-acetyl-L-cysteine–NaOH method as described by Kent and Kubica (3). A 4% concentration (starting concentration) of NaOH was used. After decontamination, smears were prepared from the concentrated sediments of the specimens for Ziehl-Neelsen (ZN) acid-fast staining. The remaining sediment was suspended in 1.5 ml of sterile phosphate-buffered saline (pH 6.8). Before inoculation, BACTEC MGIT 960 and BACTEC 12B vials were supplemented as described by the manufacturer. We inoculated 0.5 ml of the processed specimen into BACTEC MGIT 960, 0.5 ml into BACTEC 12B, and 0.2 ml onto each of two LJ medium slants. All inoculated media were incubated at 37°C. BACTEC MGIT 960 vials were introduced into the BACTEC MGIT 960 instrument as recommended by the manufacturer and tested either until they were found to be positive or for 6 weeks. The BACTEC 12B vials were read twice weekly for the first 2 weeks and weekly thereafter for 4 weeks. When the growth index of a BACTEC 12B vial reached ≥10, the vial was tested daily until the vial attained a growth index of ≥100, at which time it was considered presumptively positive. If no $^{14}CO_2$ production was detected after 6 weeks, the BACTEC 12B vial was regarded as negative. LJ medium slants were examined weekly for 8 weeks for the visible appearance of colonies. After confirmation of mycobacterial growth in a liquid or solid medium,

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TABLE 1. Rates of recovery of mycobacteria and contaminants by BACTEC MGIT 960, BACTEC 12B, and LJ medium

| Medium | No. (%) of isolates recovered[a] | | | |
|---|---|---|---|---|
| | All organisms | *M. tuberculosis* | NTM | Contaminants |
| BACTEC MGIT 960 | 55 (96.5) | 53 (96.4) | 2 (100) | 14 (3.7) |
| BACTEC 12B | 53 (93.0) | 51 (92.7) | 2 (100) | 11 (2.9) |
| LJ | 46 (80.7) | 45 (81.8) | 1 (50) | 4 (1.2) |

[a] The total number of organisms recovered was 57, of which 55 were *M. tuberculosis*, 2 were NTM, and 17 were contaminants. $\chi^2$ test for differences in recovery of mycobacteria: BACTEC MGIT 960 versus LJ medium, $P < 0.05$ (significant). $\chi^2$ test for differences in recovery of *M. tuberculosis*: BACTEC MGIT 960 versus LJ medium, $P < 0.05$ (significant).

the parallel media were read daily. On the day of detection, all positive liquid and solid media were examined by ZN staining to confirm the presence of acid-fast bacteria (AFB) and subcultured onto Columbia agar with 5% sheep blood (bioMerieux Microbiology Systems, Marcy l'Etoile, France) to check for contaminants. Cultures found AFB positive by microscopy were identified by means of the AccuProbe culture identification test (Gen-Probe, San Diego, Calif.) and conventional biochemical tests (3). The $\chi^2$ test was used to evaluate differences between recovery rates in different media. Analysis of variance (ANOVA) and the Newman-Keuls test were used to establish significant differences in relation to the duration of growth.

A total of 57 specimens (15.1%) were positive for mycobacteria, of which 14 (24.6%) were AFB smear positive and 43 (75.4%) were AFB smear negative. The mycobacterial species identified were *Mycobacterium tuberculosis* ($n = 55$), *Mycobacterium avium* complex ($n = 1$), and *Mycobacterium xenopi* ($n = 1$). The numbers of isolates of mycobacteria recovered by BACTEC MGIT 960, BACTEC 12B, and LJ medium are presented in Table 1. As a single medium, BACTEC MGIT 960 recovered 53 (96.4%) of the 55 *M. tuberculosis* isolates, BACTEC 12B recovered 51 (92.7%) of the 55 isolates, and LJ medium recovered 45 (81.8%) of the 55 isolates. A statistically significant difference was demonstrated between BACTEC MGIT 960 and LJ medium ($P < 0.05$).

In the present study, the automated 7-ml BACTEC MGIT 960 system displayed a rate of recovery (96.4%) of *M. tuberculosis* higher than those previously reported for the manual 4-ml MGIT, i.e., 89.4% by Pfyffer et al. (4), 81.3% by Somoskövi and Magyar (8), and 85% by Piersimoni et al. (5), and also higher than those previously reported for the same walk-away system, i.e., 77% by Hanna et al. (2) and 88% by Tortoli et al. (10). The 7-ml BACTEC MGIT 960 was also more efficient than the similarly fully automated ESP II system (sensitivity, 85.3 and 89%) (9, 11) and showed a sensitivity comparable to that of the MB/BacT system (96%) (1).

The lower rate of recovery observed by Hanna et al. and Tortoli et al. may have been due to a higher contamination rate with the 7-ml BACTEC MGIT 960. Hanna et al. found that after removal of the contaminated cohorts from their analysis, the sensitivity of BACTEC MGIT 960 increased from 77 to 86% (2). Contamination did not cause a serious problem in our study. The rates of contamination were 3.7, 2.9, and 1.2% for BACTEC MGIT 960, BACTEC 12B, and LJ medium, respectively (Table 1). We found that the rate of contamination with the 7-ml Bactec MGIT 960 was lower than the values reported previously for other walk-away broth-based systems (1, 2, 9, 10, 11) but was in line with the rates of contamination observed with the 4-ml MGIT (4, 5, 8). However, the concentration of NaOH used in the other walk-away system studies was lower (2%) than that used both in the present study (4%) and in the 4-ml MGIT studies (3%) (1, 2, 4, 5, 8, 9, 10, 11). Moreover, the delay between specimen collection and processing due to longer specimen shipment times at some sites (i.e., 2 to 5 days at one of the test sites in the study by Hanna et al.) and the different patient population may also explain these discrepancies (2, 4). Our laboratory usually receives the majority of samples within 30 min after collection. This rapid delivery could impact both the contamination rate and the rate of recovery.

Two *M. tuberculosis* isolates grew in the 7-ml BACTEC MGIT 960 but not on BACTEC 12B or LJ medium. BACTEC 12B or LJ medium did not detect any isolates alone. It is possible that those two isolates recovered by the 7-ml MGIT alone did not metabolize the [$^{14}$C]palmitic acid in BACTEC 12B or that the higher volume of the 7-ml BACTEC MGIT 960 diluted potential growth inhibitors in the specimen. Each system detected all 14 smear-positive specimens, and all of these contained *M. tuberculosis*. For the smear-negative specimens, the *M. tuberculosis* recovery rates were 39 (95.1%) of 41 isolates with BACTEC MGIT 960, 37 (90.2%) of 41 isolates with BACTEC 12B, and 31 (75.6%) of 41 isolates with LJ medium. Again, a statistically significant difference was found between BACTEC MGIT 960 and LJ medium ($P < 0.05$). The number of nontuberculous mycobacteria (NTM) in this study was too low to allow a meaningful statistical comparison for this group.

It is generally recommended that a solid medium not be used alone but be used in combination with a liquid-based culture system to increase the sensitivity of cultivation for mycobacteria (3, 4, 7). This combination is considered to be the "gold standard." Recovery rates were also compared when each liquid medium was combined with LJ medium. The recovery rates obtained for *M. tuberculosis* were 53 (96.4%) of 55 isolates with BACTEC MGIT 960 plus LJ medium and 51 (92.7%) of 55 isolates with BACTEC 12B plus LJ medium. The statistical analysis did not reveal any significant difference between the two combinations. Therefore, combination of the 7-ml BACTEC MGIT 960 and LJ medium could be a reliable alternative for the standard liquid radiometric plus solid medium combination. The rate of recovery of *M. tuberculosis* found in our study for the 7-ml BACTEC MGIT 960 plus solid medium was in line with the findings of 92% reported by Pfyffer et al. (4), 94.6% reported by Somoskövi and Magyar (8), and 92% reported by Piersimoni et al. (4) for the 4-ml MGIT plus solid medium; 94% reported by Woods et al. (11) and 96.1% reported by Tortoli et al. (9) for ESP II plus solid medium; and 97% reported by Hanna et al. (2) and 94% reported by Tortoli et al. (10) for the 7-ml Bactec MGIT 960 plus solid medium.

The mean (range) times to detection of all *M. tuberculosis* isolates were 14.3 (6 to 24), 16.6 (8 to 23), and 35.8 (14 to 58) days with BACTEC MGIT 960, BACTEC 12B, and LJ medium, respectively. ANOVA and the Newman-Keuls test revealed statistically significant differences between BACTEC MGIT 960 and LJ medium and between BACTEC 12B and LJ medium ($P < 0.001$ and $P < 0.001$, respectively). The difference between the two liquid media was not statistically significant. The mean times to detection of growth of all mycobacteria and *M. tuberculosis* with regard to results of acid-fast microscopy are listed in Table 2. The times to detection of *M. tuberculosis* from smear-positive specimens were comparable for the 7-ml BACTEC MGIT 960 and BACTEC 12B (12.6 versus 13.8 days). The time to detection with the 7-ml BACTEC MGIT 960 was shorter than the time of 15.3 days reported by Piersimoni et al. (5) for the manual MGIT but was

TABLE 2. Mean time to detection of all mycobacteria and *M. tuberculosis* in clinical specimens

| Medium | Mean no. of days (range) to detection[a] of: | | |
|---|---|---|---|
| | Mycobacteria | *M. tuberculosis* | |
| | | Smear positive | Smear negative |
| BACTEC MGIT 960 | 13.2 (6–24) | 12.6 (8–18) | 15.8 (6–24) |
| BACTEC 12B | 16.8 (8–23) | 13.8 (8–23) | 17.7 (9–23) |
| LJ | 36.2 (14–58) | 20.1 (14–27) | 42.2 (18–58) |

[a] ANOVA, $P < 0.001$. Newman-Keuls test for differences in mean times to detection of mycobacteria and *M. tuberculosis*: BACTEC MGIT 960 versus LJ medium, $P < 0.001$ (significant), BACTEC 12B versus LJ medium, $P < 0.001$ (significant).

longer than the time of 9.9 days observed by Pfyffer et al. (4) and the 7.2 days reported by Somosköi and Magyar (8), both for the 4-ml MGIT, and the 10.6 days reported by Hanna et al. (2) and the 12.5 days reported by Tortoli et al. (10) for the 7-ml BACTEC MGIT 960. In comparison with other walk-away systems, the time to detection of *M. tuberculosis* with the 7-ml BACTEC MGIT 960 in this study was lower than the 14.5 days found by Woods et al. (11) for ESP II but was higher than the 10.3 days given by Benjamin et al. (1) for MB/BacT. However, the proportion of smear-positive specimens was not exactly the same in all of the studies.

In our study, the mean time to detection of *M. tuberculosis* in smear-negative specimens was slightly shorter with the 7-ml BACTEC MGIT 960 than with BACTEC 12B (15.8 versus 17.2 days). Also, the time to detection with the 7-ml BACTEC MGIT 960 was shorter than those reported by Hanna et al. (18.1 days) (2), Tortoli et al. (19.6 days) (10), Pfyffer et al. (20.3 days) (4), Somosköi and Magyar (19.1 days) (8), and Piersimoni et al. (22.4 days) (5) for either the 7-ml BACTEC MGIT 960 or the manual 4-ml MGIT. The mean time to detection of *M. tuberculosis* in smear-negative specimens with the 7-ml BACTEC MGIT 960 was also much shorter than those reported for ESP II and MB/BacT, i.e., 18.9 days by Woods et al. (11) and 20.1 days by Benjamin et al. (1). Our results indicate that the automated 7-ml BACTEC MGIT 960 system may be much faster for the recovery of *M. tuberculosis* from smear-negative specimens than the manual 4-ml MGIT method and the similarly automated broth-based systems.

We did not observe any false-positive cultures (instrument positive but smear and Columbia agar subculture negative) with BACTEC MGIT 960 during the study. However, in eight BACTEC MGIT 960 vials signaled to be positive by the instrument, the confirmatory acid-fast microscopy was negative and the presence of AFB could be detected on ZN-stained smears only after incubation for 3 to 4 more days.

In summary, the recently introduced fully automated 7-ml Bactec MGIT 960 has been shown to be a viable alternative to the radiometric BACTEC 460 TB, manual 4-ml MGIT, ESP II, and MB/BacT systems for the rapid and reliable laboratory diagnosis of tuberculosis. In contrast to the BACTEC 460 TB system, it is a nonradiometric assay and there is no need for needles for the inoculation or testing of vials. Further advantages of the fully automated system include no requirement for flushing of vials prior to inoculation, manual loading of racks with vials for each test, and establishment of a reading schedule. Therefore, it is less labor-intensive and hence may free laboratory staff for other duties. In addition, the capacity of the 7-ml BACTEC MGIT 960 is much higher than that of ESP II or MB/BacT and therefore its application is more useful for laboratories dealing with large numbers of specimens daily.

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