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# Comparison of the Septi-Chek AFB and BACTEC Systems and Conventional Culture for Recovery of Mycobacteria

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**The performance of the Septi-Chek AFB system was compared with that of the BACTEC radiometric system and that of Lowenstein-Jensen agar slants (LJ) for detection of mycobacteria in clinical specimens. A total of 642 specimens were cultured; 61 (9.5%) yielded mycobacteria. *Mycobacterium tuberculosis* (34 isolates) and *Mycobacterium avium* complex (25 isolates) were the predominant species isolated. Of the 61 culture-positive specimens, 30 were smear positive and 31 were smear negative. Overall, 95% of the positive specimens were detected by Septi-Chek and BACTEC (100% of *M. tuberculosis* isolates) and 75% by LJ (82% of *M. tuberculosis* isolates). The mean times to detection were 15 days for BACTEC, 23 days for Septi-Chek, and 27 days for LJ. Of the 30 smear-positive specimens, 100% were recovered by Septi-Chek and BACTEC and 90% were recovered by LJ. Of the 31 smear-negative specimens, 90% were detected by Septi-Chek and BACTEC and 61% were detected by LJ. The Septi-Chek and BACTEC systems are superior to the conventional (LJ) mycobacterial culture method. Although Septi-Chek requires more time for the detection of mycobacteria than BACTEC, it is comparable in terms of overall recovery.**

The increase of *Mycobacterium avium* complex (MAC) infections and the resurgence of tuberculosis, especially multi-drug-resistant tuberculosis, in the United States have renewed interest in developing more-efficient systems for isolation, identification, and susceptibility testing of mycobacteria. Although the direct detection of mycobacteria in clinical specimens by molecular techniques is promising, labor-intensive methods requiring prolonged incubation remain the mainstay for most clinical laboratories. The conventional or standard method with solid media requires 3 to 6 weeks of incubation for growth of mycobacteria. The use of selective broth medium in the BACTEC 460 TB radiometric system (Becton Dickinson, Sparks, Md.) improves recovery and decreases the time required for detection of mycobacteria (2, 6) but requires capital expenditures and the disposal of radioactive waste, which preclude its use in many laboratories. The Septi-Chek AFB system (Becton Dickinson, Cockeysville, Md.) is a biphasic culture system, consisting of modified Middlebrook 7H9 broth and a three-sided paddle containing chocolate, egg-based, and modified Middlebrook 7H11 solid agars. This system does not require specialized instrumentation or the use of radioisotopes. This report summarizes a study comparing the Septi-Chek AFB system, the BACTEC 460 TB system, and a conventional culture method, Lowenstein-Jensen (LJ) medium, for recovery rates and time required for detection of mycobacteria from clinical specimens.

## MATERIALS AND METHODS

**Participants.** The study sites include the 450-bed VA Medical Center, a 120-bed VA Medical Center skilled nursing care facility, and the 350-bed Oregon Health Sciences University medical center. The VA Medical Center complex serves a predominantly male population; the Oregon Health Sciences University medical center serves both suburban and urban populations.

**Specimens.** The clinical specimens processed for isolation of mycobacteria included sputum and bronchoscopy specimens (342 specimens), urine (83 specimens), pleural fluid (32 specimens), peritoneal fluid (16 specimens), synovial fluid (9 specimens), pericardial fluid (2 specimens), cerebrospinal fluid (46 specimens), gallbladder fluid (1 specimen), aspirates (11 specimens), gastric fluid (3 specimens), drainage (4 specimens), tissue (53 specimens), stool (22 specimens), bone marrow (8 specimens), and swabs (10 specimens). Blood specimens were excluded from the study.

**Specimen processing.** Specimens were processed by standard methods (5, 5a). Sputum and other respiratory secretions were liquefied with *N*-acetyl-L-cysteine. All contaminated-site specimens were decontaminated for 15 min with NaOH (final concentration, 2%) and centrifuged for 15 min at 3,500 × *g*. Urine samples were concentrated by centrifugation at 3,500 × *g* for 15 min, and the sediment was decontaminated as described above. Normally sterile body fluids were concentrated by centrifugation at 3,500 × *g* for 30 min and could usually be cultured without prior decontamination. The sediment from all specimens was suspended in 0.067 M phosphate buffer (pH 6.8) (PB) to a final volume of 2.0 ml. Tissue specimens were processed with a tissue grinder or stomacher (Tekmar Co., Cincinnati, Ohio) in sterile 0.9% NaCl and adjusted to a final volume of 2.0 ml with PB. Smears were prepared from all specimens for acid-fast staining.

**Media and culturing methods.** Samples (0.5 ml) of specimens were inoculated onto each culture medium. Each of two LJ agar slants was inoculated with 0.25 ml of specimen. One slant of selective LJ medium was substituted for a nonselective LJ slant when contaminated-site specimens were cultured. All media were incubated at 35°C in 7.5% $CO_2$ and inspected weekly for 8 weeks. After inoculation, the Septi-Chek system was inverted, allowing the broth to wash over the agar surfaces, and incubated upright at 37°C.

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TABLE 1. Distribution of 61 mycobacterial isolates from clinical specimens

| Type of specimen (n) | No. of isolates recovered with: | | |
|---|---|---|---|
| | LJ | BACTEC | Septi-Chek |
| Respiratory secretions[a] (342) | 42 | 51 | 51 |
| Body fluid (117) | 1 | 2 | 2 |
| Tissue (53) | 0 | 1 | 1 |
| Stool (22) | 3 | 4 | 4 |
| Others[b] (108) | 0 | 0 | 0 |
| Total (642) | 46 | 58 | 58 |

[a] Sputum and bronchoscopy specimens.
[b] See Materials and Methods for specific specimen type.

Growth in the Septi-Chek broth was detected by visual inspection, and smears were made when flakes, granules, or increased turbidity was observed in the fluid broth. The BACTEC medium was processed as recommended by the manufacturer. Smears were made when the growth index reached 20 or greater. Both the Septi-Chek and the BAC TEC systems were inspected for growth three times per week for the first 2 weeks and weekly thereafter for an additional 6 weeks. All systems were inspected independently for growth. The contamination rates of the systems were 3.7% (Septi-Chek), 5.5% (BACTEC), and 7.0% (LJ). Mycobacterial isolates were identified by conventional methods employed routinely in the laboratory and/or with a mycobacterial probe (Gen-Probe Inc., San Diego, Calif.).

**Statistical analysis.** The isolation rates of the three systems were evaluated by the McNemar modification of the $\chi^2$ test (8).

## RESULTS

A total of 642 specimens were included in the study, of which 61 (9.5%) yielded mycobacteria (Table 1). Of these 61 cultures, 34 grew *Mycobacterium tuberculosis*, 25 yielded MAC, and 2 grew *Mycobacterium gordonae*. Of the 61 culture-positive specimens, 30 (49.2%) were acid-fast smear positive and 31 (50.8%) were acid-fast smear negative. More than 90% of the isolates were recovered from sputum and bronchoscopy specimens.

Table 2 summarizes the rate of recovery of mycobacteria by each culture system. Both BACTEC and Septi-Chek had an overall detection rate of 95%, compared with 75% for the solid (LJ) medium ($P < 0.01$). BACTEC and Septi-Chek were significantly better at isolating *M. tuberculosis* and MAC organisms than the conventional solid medium ($P < 0.05$). In the biphasic Septi-Chek system, mycobacteria were recovered equally on the three-sided paddle containing three solid media and in the broth. On LJ medium, *M. tuberculosis* showed a higher degree of recovery than MAC (82 versus 68%). No difference was observed in the recovery of *M. gordonae* by the three systems. However, the number of isolates was too small to evaluate.

TABLE 2. Rates of recovery of mycobacteria by three culture methods

| Species (no. of isolates) | No. (%) of isolates recovered with: | | |
|---|---|---|---|
| | LJ | BACTEC | Septi-Chek |
| *M. tuberculosis* (34) | 28 (82.4) | 34 (100) | 34 (100) |
| MAC (25) | 17 (68.0) | 23 (92) | 23 (92) |
| *M. gordonae* (2) | 1 (50.0) | 1 (50) | 1 (50) |
| Total (61) | 46 (75.4) | 58 (95.1) | 58 (95.1) |

TABLE 3. Rates of recovery of mycobacteria from smear-positive and smear-negative specimens

| Smear result (no. of isolates) | No. (%) of isolates recovered with: | | |
|---|---|---|---|
| | LJ | BACTEC | Septi-Chek |
| Positive (30) | 27 (90.0) | 30 (100) | 30 (100) |
| Negative (31) | 19 (61.3) | 28 (90.3) | 28 (90.3) |
| Total (61) | 46 (75.4) | 58 (95.1) | 58 (95.1) |

A comparison of the mycobacterial recovery rates of the three methods in combination did not produce data materially different from those shown in Table 2. The combination of LJ and BACTEC yielded one additional isolate (*M. gordonae*), while no additional isolates were detected by the combination of LJ and Septi-Chek. BACTEC and Septi-Chek together detected 100% of the mycobacterial species. The LJ system alone did not detect any isolates missed by the other two systems. BACTEC alone detected two MAC isolates and one *M. gordonae* isolate, while two MAC isolates were detected only by Septi-Chek.

The rates of recovery of mycobacteria from smear-positive and smear-negative specimens are presented in Table 3. Septi-Chek and BACTEC detected all isolates from smear-positive specimens, compared with 90% for LJ medium ($P > 0.2$). BACTEC and Septi-Chek were significantly better at recovering isolates from smear-negative specimens, isolating 90% compared with 61% isolated by conventional solid medium ($P < 0.02$).

The average number of days required for the recovery of mycobacteria by each culture system is shown in Tables 4 and 5. The overall mean times to detection were 15 days for BACTEC, 23 days for Septi-Chek broth, 29 days for Septi-Chek solid media (paddle), and 27 days for LJ (data not shown). A comparison of the isolation times for mycobacteria recovered from smear-positive specimens showed that BACTEC detected *M. tuberculosis* and MAC growth earlier than Septi-Chek or LJ (Table 4). On the average, BACTEC detected MAC isolates 6 days earlier than *M. tuberculosis* isolates. This difference was only 1 to 2 days for LJ and Septi-Chek. Septi-Chek detected growth for both mycobacterial species earlier than LJ. Growth occurred as early as 4 to 7 days after inoculation of the medium and as late as 32 to 39 days in the BACTEC and Septi-Chek systems.

The average times for detection of mycobacteria from smear-negative specimens were 18 days for Bactec, 27 days for Septi-Chek, and 32 days for LJ, 6 to 8 days longer than the times observed for smear-positive specimens (Table 5).

TABLE 4. Average times for detection of mycobacteria from smear-positive specimens[a]

| Method | Avg no. of days to detection (range) of: | | |
|---|---|---|---|
| | *M. tuberculosis* | MAC | All isolates |
| LJ | 23.9 (13–38) | 22.3 (17–29) | 23.5 (13–38) |
| BACTEC | 13.1 (4–32) | 7.4 (4–14) | 11.8 (4–32) |
| Septi-Chek | 19.2 (9–39) | 17.6 (7–29) | 18.8 (7–39) |

[a] Data are based on positive cultures only.

TABLE 5. Average times for detection of mycobacteria from smear-negative specimens[a]

| Method | Avg no. of days to detection (range) of: | | | |
|---|---|---|---|---|
| | *M. tuberculosis* | MAC | *M. gordonae* | All isolates |
| LJ | 21.7 (14–33) | 39.6 (25–56) | 22 | 32.1 (14–56) |
| BACTEC | 21.8 (11–40) | 15.3 (6–34) | 13 | 17.8 (6–40) |
| Septi-Chek | 36.5 (14–58) | 20.3 (8–53) | 36 | 27.3 (8–58) |

[a] Data are based on positive cultures only.

MAC isolates were detected earlier than *M. tuberculosis* by the Septi-Chek and BACTEC systems. The mean times to detection of *M. tuberculosis* were 22 days for LJ and 37 days for Septi-Chek. However, the average times to detection were similar (22 days) for both of these systems when specimens found positive by Septi-Chek only were excluded (data not shown).

## DISCUSSION

The increase in tuberculosis and infections due to MAC requires faster and more efficient methods that can be used by a greater number of clinical microbiology laboratories. This study compares the isolation rates of two liquid media (BACTEC and Septi-Chek media) with that of conventional solid medium (LJ medium). Our data confirm previous findings that the BACTEC and Septi-Chek systems are significantly superior to conventional solid media for the isolation of *M. tuberculosis* and MAC organisms from all clinical specimens (1, 3, 4, 6, 7, 9). There was no statistical difference in the rates of recovery of mycobacteria with BACTEC and Septi-Chek, confirming the results of earlier studies (1, 6, 7). This observation contrasts with that of Whittier et al. (9), who reported that Septi-Chek was significantly better than BACTEC for the isolation of mycobacteria. A difference in the recovery rates between the liquid media (BACTEC and Septi-Chek media) and the solid medium (LJ medium) was observed for both smear-positive and smear-negative specimens. However, this difference was statistically significant ($P < 0.02$) only for smear-negative specimens, consistent with the findings of Abe et al. (1). These data suggest that liquid media enhance the recovery of small numbers of mycobacteria from clinical specimens.

The recovery of *M. tuberculosis* from smear-positive specimens by BACTEC occurred 6 and 11 days earlier than isolation by Septi-Chek and LJ, respectively. A greater difference in the mean time to detection was observed for MAC isolates. Septi-Chek exhibits faster recovery times than LJ for both mycobacterial species. These results are similar to data reported in other studies (1, 3, 6, 7, 9). However, the biphasic Septi-Chek system and conventional solid media provide isolated colonies earlier than the BACTEC system.

The time for detection of *M. tuberculosis* from smear-negative specimens by LJ medium was the same as that by BACTEC and 15 days earlier than that by Septi-Chek, contrasting with other reports (1, 3). Our data were calculated from all positive specimens. Four *M. tuberculosis* isolates recovered only in BACTEC and Septi-Chek averaged 30 and 57 days to detection, respectively. Excluding these isolates from the data produces average detection times of 17 days for BACTEC and 22 days for both Septi-Chek and LJ.

The Septi-Chek and BACTEC systems are comparable in terms of overall recovery and are superior to conventional culture methods (LJ) for recovery and time to detection of mycobacterial growth. BACTEC detects growth of mycobacteria earlier than Septi-Chek, but Septi-Chek does not require specialized equipment.

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