Evaluation of BacT/Alert 3D Liquid Culture System for Recovery of Mycobacteria from Clinical Specimens Using Sodium Dodecyl (Lauryl) Sulfate-NaOH Decontamination

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A total of 52 mycobacterial isolates were recovered from 1,197 clinical specimens decontaminated by a sodium dodecyl (lauryl) sulfate (SDS)-NaOH protocol. Of these, 94% were recovered with the BacT/Alert 3D system (Organon Teknika, Durham, N.C.) and 79% were recovered on Löwenstein-Jensen (LJ) medium. Mean times to detection of organisms of the Mycobacterium tuberculosis complex (n = 47) were 22.8 days with LJ medium and 16.2 days with the system. The BacT/Alert 3D system is a rapid and efficient detection system which can be used with an SDS-NaOH decontamination procedure.

Rapid and sensitive detection of Mycobacterium tuberculosis is of clinical importance for the treatment, control, and prevention of tuberculosis. Despite new nucleic acid amplification assays, unequivocal diagnosis of tuberculosis continues to rely on cultivation of M. tuberculosis. The BacT/Alert 3D system (Organon Teknika, Durham, N.C.) is a fully automated liquid culture system which allows the growth and detection of mycobacteria. Further studies indicate that the BacT/Alert 3D system is a rapid and sensitive method for recovery of mycobacteria from clinical specimens using an N-acetyl-l-cysteine-NaOH decontamination method (1, 2, 5, 9). Many laboratories, particularly in European countries, pretreat their respiratory specimens by sodium dodecyl (lauryl) sulfate (SDS)-NaOH decontamination. The aim of this study was to compare the BacT/Alert 3D system with the use of egg-based Löwenstein-Jensen (LJ) solid medium, using the SDS-NaOH decontamination method in a routine mycobacteriology laboratory procedure.

A total of 1,197 clinical specimens (668 bronchoalveolar lavage fluid specimens, 247 sputum specimens, 56 gastric aspirate specimens, 26 urine specimens, 8 pleural fluid specimens, 32 cerebrospinal fluid specimens, 61 abscess specimens, 85 tissue biopsy specimens, 2 peritoneal fluid specimens, 4 pericardial fluid specimens, 2 ascitic fluid specimens, and 6 synovial fluid specimens) from 980 patients were tested from 1 December 1999 to 1 December 2000. The specimens were processed according to standard protocols (4). Tissues were homogenized and crushed. Respiratory specimens, urine, and contaminated-site specimens were decontaminated with SDS-NaOH according to the method described by Tacquet and Tisson (8), with a supplementary washing step. Briefly, 3 ml of the specimen was transferred to a 50-ml plastic centrifuge tube, and an equal volume of SDS-NaOH solution (1% NaOH, 3% SDS) was added. After vortexing, the samples were vigorously shaken for 30 min. H2PO4 containing 0.006% bromocresol purple as a pH indicator was added to neutralize the specimen. After a centrifugation step (4,000 × g for 20 min), the pellet obtained was washed in 40 ml of distilled water and resuspended in 1.5 ml of distilled water. A small amount of sediment was used to prepare smears for auramine fluorochrome staining. Slides that were positive for acid-fast bacilli were confirmed by Ziehl-Neelsen staining. The same amount of each concentrated sample (0.5 ml) was inoculated either into vials of the BacT/Alert 3D system containing modified Middlebrook 7H9 with an antibiotic supplement (ampicillin [0.018%, wt/vol], azlocillin [0.0034%, wt/vol], nalidixic acid [0.04%, wt/vol], trimethoprim [0.00105%, wt/vol], polymyxin B [10,000 U], and vancomycin [0.0005%, wt/vol]) or onto two LJ slants (0.25 ml each). All mycobacterial cultures were incubated at 37°C. The LJ slants were inspected weekly for growth over an 8-week period. BacT/Alert 3D vials were monitored continuously by the BacT/Alert 3D system. Nonmycobacterial growth was detected using blood agar plates. Growth of mycobacteria was verified by microscopy (Ziehl-Neelsen staining). Conventional biochemical techniques or Accu-Probe M. tuberculosis complex identification tests (Gen-Probe; bioMérieux, Marcy l’Etoile, France) were used to identify the isolates (4).

From a total of 1,197 specimens, mycobacteria were identified in 52 cultures (4.3%). Recovery rates for all mycobacteria were 94 and 79%, respectively, for the BacT/Alert 3D system and LJ medium (Table 1). Recovery rates for the M. tuberculosis complex were 94 and 85%, respectively, for the BacT/Alert 3D system and LJ medium. Twelve isolates grew only on a single medium. Nine strains were detected with the BacT/Alert 3D system alone: five M. tuberculosis complex strains and four nontuberculous mycobacteria. Three M. tuberculosis complex strains were detected with LJ medium alone. This underscores the need to use the combination of liquid and solid media as recommended (4). The mean times to detection of the M. tuberculosis complex were 22.8 days with LJ medium and 16.2 days with the BacT/Alert 3D system. The average numbers of days required for detection of M. tuberculosis with regard to microscopy were as follows. The average time to detection of the M. tuberculosis complex with the BacT/Alert 3D system was 14.3 days (ranging from 6 to 24 days) for 13 smear-positive isolates and 17.4 days (ranging from 7 to 35 days) for 24 smear-
TABLE 1. Isolation of mycobacteria with the BacT/Alert 3D system and LJ medium

<table>
<thead>
<tr>
<th>Organism (no. of isolates)</th>
<th>No. (%) of isolates recovered with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BacT/Alert 3D system</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis complex (47)</td>
<td>44 (94)</td>
</tr>
<tr>
<td>Mycobacterium avium complex (2)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Mycobacterium malmoense (2)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Mycobacterium gordoniae (1)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Total (52)</td>
<td>49 (94)</td>
</tr>
</tbody>
</table>

negative isolates. The average time to detection of the *M. tuberculosis* complex with LJ medium was 19.4 days (ranging from 13 to 30 days) for 13 smear-positive isolates and 24.6 days (ranging from 16 to 35 days) for 24 smear-negative isolates. The contamination rates (95% bacterial origin) for the BacT/Alert 3D system and for LJ medium were 6.2 and 4.6%, respectively.

The contamination procedure, the recovery rates for mycobacteria isolated from clinical specimens, especially in Europe, had not been evaluated previously. We therefore studied the possible utilization of this contamination method with the BacT/Alert 3D system using a supplementary washing step after the SDS-NaOH decontamination. Other studies have demonstrated that this washing step is sufficient to remove all remaining traces of detergent that could interfere with the results (3, 6, 7). Using this decontamination procedure, the recovery rates for mycobacteria isolated in this study in the BacT/Alert 3D system compared to those of all mycobacteria recovered using either solid or liquid media (94%) were equivalent to those found by other authors (95.3% for Palacios et al. [5], 96.5% for Brunello et al. [2], and 91.6% for Yan et al. [9]) using the MB/BacT system (same vials and same algorithm system as the BacT/Alert 3D system) and an N-acetyl-L-cysteine-NaOH decontamination method. Similarly, the average number of days required for the detection of *M. tuberculosis* complex strains (14.3 days for smear-positive specimens and 17.4 days for smear-negative specimens) is comparable to those described by other authors (1, 2, 5, 9).

These results indicate that pretreatment of clinical specimens using SDS-NaOH with a supplementary washing step could be used for recovery of the *M. tuberculosis* complex with the BacT/Alert 3D system.

REFERENCES