Recovery and Susceptibility Testing of *Mycobacterium tuberculosis* from Extrapulmonary Specimens by the BACTEC Radiometric Method

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This study was carried out to evaluate the sensitivity and rapidity of the BACTEC radiometric techniques for isolation and susceptibility testing of mycobacteria from extrapulmonary specimens. Concentrated specimens of urine, pleural fluid, and blood as well as other extrapulmonary specimens were processed for the recovery of mycobacteria and for drug susceptibility testing, employing conventional and BACTEC radiometric methods. Of 483 specimens processed, 20 were found to be positive for *Mycobacterium tuberculosis* on the conventional Lowenstein-Jensen medium, and 19 were found to be positive in the BACTEC 7H12 medium. Average recovery times were 22.5 days for the conventional method and 10.9 days for the BACTEC method. When isolated cultures were tested for susceptibility to streptomycin, isoniazid, rifampin, and ethambutol, results were reported at an average time of 22 and 5.4 days for the conventional and BACTEC methods, respectively, with good correlation.

Several reports have been published on the detection and recovery as well as susceptibility testing of *Mycobacterium tuberculosis* from sputum specimens by the radiometric method (1, 3–6). However, no report dealing with the use of radiometric techniques for extrapulmonary specimens has been published. Since specimens other than sputa are also frequently submitted for mycobacterial investigations, we decided to evaluate the BACTEC rapid radiometric technique for these samples. In this study, concentrated specimens of urine, pleural fluid, and blood as well as other nonsputum samples were examined by using conventional Lowenstein-Jensen (L-J) slants and the new BACTEC Middlebrook 7H12 liquid (12A) medium (Johnston Laboratories, Towson, Md.). There were 408 urine (early morning void), 11 cerebrospinal fluid, 26 pleural fluid, 8 blood, and 30 miscellaneous fluid specimens analyzed by the two methods.

Aseptically collected specimens were centrifuged at 2,500 × g for 15 min, washed with 10 ml of phosphate-buffered saline (pH 7.5), and suspended with 0.2% fatty acid-free albumin. Nonsterile specimens were decontaminated by using 2% NaOH for 15 min and then processed in the same manner.

After processing and concentrating the specimen, 0.1 to 0.2 ml of the sample was inoculated onto two L-J slants, and 0.1 to 0.2 ml was inoculated into a 7H12 vial which contained 2 ml of medium. The 7H12 medium was supplemented with 0.1 ml of PACT solution (Johnston Laboratories). This is a mixture of antimicrobials used to reduce nonmycobacterial contamination. The final concentrations of antimicrobials in 7H12 medium were (per milliliter): polymixin B, 50 U; amphotericin B, 5 μg; carbenicillin, 25 μg; and trimethoprim, 2.5 μg. Both L-J and 7H12 cultures were incubated at 36 to 38°C with 5% CO2 for a maximum of 6 weeks. The L-J slants were examined weekly for visible colonies or were examined at the time when 7H12 was positive. BACTEC 7H12 vials were read daily on the BACTEC 460 TB instrument (Johnston Laboratories) for detection and quantitation of 14CO2 produced during the growth of mycobacteria and metabolism of 14C-labeled substrate present in the medium.

The auramine-rhodamine and Ziehl-Neelsen staining procedures were used to confirm the presence of acid-fast bacteria in positive cultures. Identification was carried out by conventional methods (2).

Isolated cultures from L-J slants were then tested for susceptibility to streptomycin (S), isoniazid (I), rifampin (R), and ethambutol (E). The conventional method used was that of Wayne and Krasnow (7), with paper disks used to yield final concentrations in 7H12 medium of (micrograms per milliliter): S, 2 and 10; I, 0.2 and 1; R, 1 and 2; and E, 5 and 10 (BBL Microbiology Systems, Cockeysville, Md.).

Culture-positive BACTEC 7H12 vials at growth index 600 to 900 were used for inoculation in the BACTEC drug susceptibility test, which is a modified version of the proportion method (4). The 1% threshold for susceptibility is achieved by using a bacterial inoculum in the control that is 1/100th of that used in the drug-containing vials. The daily increase in 14CO2 production in the control and drug vials is compared to assess the drug susceptibility. The drug concentrations used with the BACTEC in 7H12 medium were (micrograms per milliliter): S, 4; I, 0.2; R, 2; and E, 10.

A total of 483 specimens were analyzed in this study, and 12 smears (2.5%) were positive for acid-fast bacteria. A total of 20 specimens (4.1%) from 20 patients were found to be culture positive by L-J, and 19 (3.9%) were found to be culture positive by BACTEC (Table 1). The average recovery times for the conventional and BACTEC systems were 22.5 days (range, 8 to 40 days) and 10.9 days (range, 5 to 30 days), respectively. No mycobacteria other than *M. tuberculosis* were recovered in this study.

In comparing the susceptibility results of the two methods, there were 95% agreement determinations with S and I and 100% agreement determinations with E and R (Table 2). Two strains were resistant to S (2 and 10 μg/ml) and I (0.2 and 1.0 μg/ml) by the conventional method, and only one out of these two was resistant to both drugs by the radiometric method. Resistance to R was shown by four strains in both methods (two strains were also resistant to S and I with the conventional method). No E-resistant strains were...
TABLE 1. *M. tuberculosis* isolated from extrapulmonary specimens by conventional and radiometric methods

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No. recovered</th>
<th>L-J</th>
<th>7H12</th>
<th>Agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>16</td>
<td>15</td>
<td>1</td>
<td>93.8</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Blood</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

a Total agreement, 95%.

found. The average time to report the results was 22 days (range, 18 to 25 days) and 5.4 days (range, 3 to 7 days) for the conventional and BACTEC methods, respectively. Since the number of resistant strains tested in this study was so small, it is not possible to compare the data with the previous studies or to draw any conclusions.

Although the total number of positive cultures was low in this study, it is evident from the results that the positivity rates of extrapulmonary specimens by the conventional and BACTEC methods are closely comparable. Only one specimen of urine, which was smear negative and had only two colonies on L-J slants, was missed by the BACTEC method. This occurred early in the evaluation period, and lack of experience could also contribute to this difference. As far as the rapidity of the system is concerned, overall results for both recovery and susceptibility testing of *M. tuberculosis* were reportable in an average of 44.5 days by the conventional method and 17.3 days by the BACTEC method, with a time savings of 27 days.

This study indicates that the radiometric method can be used for clinical specimens other than sputum for recovery of *M. tuberculosis*. The use of both 7H12 and L-J media gives optimal recovery and significantly reduces the time required to report positive results.

LITERATURE CITED


