Cord Formation in BACTEC 7H12 Medium for Rapid, Presumptive Identification of Mycobacterium tuberculosis Complex

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We evaluated cord formation in BACTEC 7H12 medium as a criterion for rapid identification of Mycobacterium tuberculosis complex. Kinyoun-stained smears, prepared from 270 radiometrically positive BACTEC 7H12 bottles, were examined independently by three observers. Smears from 93.2, 88.6, and 83.0% of the M. tuberculosis complex cultures were read as cord positive, and smears from 97.3, 97.8, and 99.5% of the mycobacteria other than M. tuberculosis cultures were read as cord negative by the three observers, respectively. There was 93.3% agreement between the observers. The presence of cords in BACTEC 7H12 medium can be a reliable criterion for rapid, presumptive identification of M. tuberculosis complex.

Virulent strains of Mycobacterium tuberculosis complex often grow as characteristic ropes, bundles or serpentine cords of acid-fast bacilli in liquid media (6). Avirulent variants of M. tuberculosis and most mycobacteria other than M. tuberculosis (MOTT) grow in liquid media in a nonoriented, dispersed fashion. The ability of M. tuberculosis to grow in vitro as serpentine cords is influenced by the composition of the culture medium and the conditions of culture (5). Gilkerson et al. (2) reported finding distinctive microscopic morphology in cover glass cultures of various mycobacteria. Lorian (5) found that the presence of typical cords on Middlebrook 7H10 agar with Triton WR-1339 can be used to differentiate M. tuberculosis complex from MOTT, except for Runyon Group I strains.

The BACTEC radiometric system for mycobacteria (Johnston Laboratories, Towson, Md.) has substantially reduced the time required to isolate and identify mycobacteria and has found widespread use in clinical laboratories (1, 4, 8, 13). The manufacturer suggests that careful observation of the morphology of acid-fast bacilli in the smears from positive BACTEC 7H12 bottles may help in the differentiation of M. tuberculosis complex from MOTT (12). Roberts et al. (11) evaluated the BACTEC radiometric method for recovery of mycobacteria from clinical specimens and noted that morphology of mycobacteria on smears could tentatively differentiate M. tuberculosis complex from MOTT, but the data was not given.

In this study, we assessed the feasibility of rapidly differentiating M. tuberculosis complex from MOTT on the basis of the ability of the former to grow as serpentine cords in BACTEC 7H12 medium. We found cord formation to be a reliable criterion for rapid, presumptive identification of M. tuberculosis complex.

This material was presented in part at the 89th Annual Meeting of the American Society for Microbiology (P. Yagupsky, D. Kaminski, K. Palmer, and F. Nolte. Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, U-31, p. 160). Tissue and normally sterile body fluids were inoculated directly into culture medium. All other specimens were first processed by the NaOH-\(N\)-acetyl-L-cysteine method and then concentrated by centrifugation (14). Specimens were cultured by using one BACTEC 7H12 bottle and one Lowenstein-Jensen agar slant (BBL Microbiology Systems, Cockeysville, Md.). BACTEC bottles were incubated at 35°C and were read three times a week for the first 3 weeks and once weekly for the remaining 3 weeks. The agar slants were incubated for 8 weeks at 35°C in an atmosphere of 5 to 10% \(CO_2\). When the growth index value exceeded 100, smears were made and stained with Kinyoun acid-fast stain, and the 7H12 broth was subcultured on Lowenstein-Jensen agar. Samples from Kinyoun-positive bottles without evidence of bacterial contamination were inoculated into a p-nitro-alpha-acetyl-amino-beta-hydroxypropionophenone (NAP) test bottle (7). Final identification of all mycobacteria was determined with conventional biochemical tests (14).

Kinyoun acid-fast-stained smears from positive BACTEC vials were saved and examined independently by three observers for the presence of serpentine cords of acid-fast bacilli (AFB). Serpentine cords were defined as ropelike aggregates of AFB in which the long axes of the bacteria paralleled the long axis of the cord. The observers were not aware of the results of the final identification of the mycobacteria or of the interpretations of the smear by the other observers. The observers were a postdoctoral resident in clinical microbiology, the mycobacteriology laboratory supervisor, and a senior medical technologist. The assessment of the cord formation in BACTEC 7H12 medium was compared with the results of the final biochemical identifications separately for each observer.

Kinyoun-stained slides prepared from 270 radiometrically positive cultures were examined for the presence of cords of AFB. Clinical specimens included 186 sputum, 23 bronchial wash/lavage, 22 tissue, 14 urine, 10 stool, 8 bone marrow, and 7 normally sterile body fluid (three cerebrospinal, two pleural, one pericardial, and one joint fluid) samples. A total of 88 cultures obtained from 31 patients grew M. tuberculosis complex, and 182 cultures from 122 patients grew MOTT (Table 1). Positive cultures were detected radiometrically after a mean ± standard deviation of 14.3 ± 7.9 days for M. tuberculosis complex and 17.6 ± 14.5 days for MOTT. The

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median growth index at the time smears were made was 192 for *M. tuberculosis* complex and 197 for MOTT.

Smears from 93.2, 88.6, and 83.0% of the *M. tuberculosis* complex cultures were read as cord positive and smears from 97.3, 97.8, and 99.5% of the MOTT were read as cord negative by the three observers, respectively. The results of the smear examination by the three observers are given in Table 2. The independent observations of observer 1 and observer 2 correlated closely, while observer 3 was more conservative in assessing cord formation. Overall, there was 93.3% agreement among the three observers.

Four of the six smears that were read as cord positive by observers 1 and 2 but were not *M. tuberculosis* were prepared from cultures containing *M. kansasii*, and the remaining two were identified as *M. avium* complex. Typical microscopic morphologies of *M. tuberculosis* and *M. kansasii* in 7H12 medium are shown in Fig. 1. In general, MOTT grew in 7H12 medium as dispersed single cells or as small clumps of bacilli with a random orientation. However, 1 of 126 (0.8%) *M. avium* complex cultures and 2 of 6 (33.3%) cultures that grew *M. kansasii* were interpreted as cord positive by two observers. Figure 1B shows the arrangement of the bacilli seen in a culture of *M. kansasii* and illustrates the difficulty in distinguishing the organized clumps from the serpentine cords of *M. tuberculosis* shown in Fig. 1A. In retrospect, the one *M. avium* complex culture that was read as cord positive contained too many organisms per microscope field to adequately assess the arrangement of the individual bacilli. Generally, those cultures of *M. tuberculosis* that showed no evidence of cord formation had few bacilli present in the AFB smear. Smears were scored as equivocal if the aggregates of mycobacteria did not meet the criteria for cords. One culture of *M. avium* complex was scored as equivocal by observer 1, and one culture of *M. kansasii* was scored as equivocal by all three observers. The average sensitivity, specificity, and positive and negative predictive values of the cord criterion for the presumptive identification of *M. tuberculosis* complex were 88.3, 99.6, 97.6, and 94.6%, respectively. The performance characteristics were calculated by considering negative and equivocal results together.

The rapid differentiation of *M. tuberculosis* complex from MOTT has important therapeutic and epidemiologic implications. Gas-liquid chromatography, NAP growth inhibition, and nucleic acid hybridization methods have all been used to shorten the time required to identify mycobacteria. Careful examination of the morphology and orientation of AFB in smears from positive cultures can also rapidly differentiate *M. tuberculosis* from MOTT.

Our data show that cord formation in BACTEC 7H12 medium can be a reliable criterion for presumptive identification of *M. tuberculosis* complex. Depending upon the observer, from 8.0 to 17.0% of cultures positive for *M. tuberculosis* complex did not form cords in this medium. However, the positive predictive values for cord formation for each observer were excellent and ranged from 96.3 to 100%. In our study, two cultures of *M. kansasii* were found to form very loose and incomplete cords by two observers. Cord formation by *M. kansasii* has been previously de-

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TABLE 1. Mycobacteria isolated from 270 positive BACTEC 7H12 cultures

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of:</th>
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<tbody>
<tr>
<td></td>
<td>Cultures</td>
</tr>
<tr>
<td><em>M. avium</em> complex</td>
<td>126</td>
</tr>
<tr>
<td><em>M. tuberculosis</em></td>
<td>86</td>
</tr>
<tr>
<td><em>M. xenopi</em></td>
<td>43</td>
</tr>
<tr>
<td><em>M. kansasii</em></td>
<td>6</td>
</tr>
<tr>
<td><em>M. gordoniae</em></td>
<td>3</td>
</tr>
<tr>
<td><em>M. bovis</em></td>
<td>2</td>
</tr>
<tr>
<td><em>M. terrae</em> complex</td>
<td>1</td>
</tr>
<tr>
<td><em>M. fortuitum</em></td>
<td>1</td>
</tr>
<tr>
<td><em>M. xenopi</em> and <em>M. gordoniae</em></td>
<td>1</td>
</tr>
<tr>
<td><em>M. avium</em> complex and <em>M. scrofulaceum</em></td>
<td>1</td>
</tr>
</tbody>
</table>

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TABLE 2. Cord formation for differentiation of *M. tuberculosis* complex from MOTT by observer

<table>
<thead>
<tr>
<th>Cord formation</th>
<th>No. of stained smears identified as:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>M. tuberculosis</em> complex (n = 88) by observer:</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Positive</td>
<td>82</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
</tr>
<tr>
<td>Equivocal</td>
<td>0</td>
</tr>
</tbody>
</table>

* Final identification, two *M. kansasii* and one *M. avium* complex.
* Final identification, two *M. kansasii* and one *M. avium* complex.
* Final identification, one *M. kansasii* and one *M. avium* complex.
* Final identification, *M. avium* complex.

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FIG. 1. Microscopic morphology of *M. tuberculosis* (A) and *M. kansasii* (B) grown in BACTEC 7H12 broth. Kinyoun acid-fast stain (magnification, ×950).
scribed (2), but the microscopic morphology of these cords differed from the true cords formed by human tubercle bacilli and with additional experience probably could be differentiated from cords formed by M. tuberculosis complex. Cord formation has also been observed in cultures of M. phlei and M. terrae (10), but in general liquid cultures of MOTT show no cording and the bacilli are random in orientation.

Cord formation was easily and reproducibly assessed in stained smears prepared from positive BACTEC bottles, and presumptive identification of M. tuberculosis on the basis of microscopic morphology was available, on average, 6 days earlier than the presumptive identification provided by the NAP differentiation test. During the study, 174 NAP differentiation tests were done, and three (1.7%) cultures were incorrectly identified as M. tuberculosis complex. Conventional biochemical testing identified two of these cultures as M. xenopi and one as M. kansasii. The two M. xenopi cultures did not form cords, whereas the M. kansasii smear was read as cord positive by two observers.

In laboratories using DNA probes (3, 9) for rapid identification of mycobacteria recovered in BACTEC bottles, microscopic morphology can be assessed in bottles with lower growth indices (>100 versus >999) and could also guide the selection of the appropriate probe for culture confirmation. Regardless of the method used to identify mycobacteria, the careful examination of microscopic morphology in 7H12 broth is a useful first step in the identification procedure.

The mycobacteria included in our study are representative of organisms commonly encountered in many clinical laboratories. However, the numbers of strains of some MOTT were small and many species were not represented. Given these limitations, we have demonstrated that cord formation in BACTEC 7H12 medium can be a reasonably sensitive and highly specific criterion for the presumptive identification of M. tuberculosis complex, and we urge other laboratories to develop their own experience.

LITERATURE CITED