Pretreatment of Clinical Specimens with Sodium Dodecyl (Lauryl) Sulfate Is Not Suitable for the Mycobacteria Growth Indicator Tube Cultivation Method

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Received 14 January 1997/Returned for modification 4 March 1997/Accepted 25 April 1997

When using the Mycobacteria Growth Indicator Tube (MGIT), pretreatment of clinical specimens with N-acetyl-l-cysteine-NaOH is recommended by the manufacturer. Processing of clinical specimens (n = 1,000) with sodium dodecyl (lauryl) sulfonate-NaOH resulted in both poor recovery and delayed mean time to detection of acid-fast bacilli. Values were comparable to those obtained on solid media.

Recently, a hitherto-nonautomated new culture technique, the Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson Microbiological Systems, Cockeysville, Md.), has been developed to circumvent commonly observed problems associated with radiometric detection systems, such as the potential danger of needle puncture and the accumulation of radioactive waste. The MGIT, which contains modified Middlebrook 7H9 broth in conjunction with a fluorescence quenching-based oxygen sensor, can be used for both rapid detection of acid-fast bacilli (AFB) (3) and susceptibility testing (7). Zuhre Badak et al. (10) reported rates of recovery of 90 and 82% for Mycobacterium tuberculosis and M. avium complex, respectively, by this system, in contrast to 93 and 85% by the BACTEC 460 (BACTEC 460 TB system; Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.). A recent European multicenter study including 1,500 clinical specimens which had been pretreated with N-acetyl-l-cysteine (NALC)-NaOH demonstrated that there was no statistically significant difference (P > 0.05) between a combination of the MGIT and solid media and a combination of the BACTEC 460 and solid media in detecting mycobacteria (6). The mean times to detection of M. tuberculosis complex were 9.9 days with the MGIT, 9.7 days with the BACTEC 460, and 20.2 days with solid media, while on average, 11.9, 13.0, and 22.2 days were needed for nontuberculous mycobacteria (NTM) to appear when these three methods were used.

According to the manufacturer of the MGIT, clinical specimens have to be pretreated with NALC-NaOH (2). At present, little is known about the efficacy of the new cultivation technique when specimens have been pretreated by procedures other than the NALC-NaOH method. In two independent studies, sodium dodecyl (lauryl) sulfonate (SDS)-NaOH, which is less commonly applied in the United States than in European countries, has proven to be an efficient alternative in eradicating rapidly growing contaminants in clinical specimens (1, 9). A modified SDS-NaOH procedure yielded even higher rates of detection of mycobacteria than did NALC-NaOH in the BACTEC 460 and on Löwenstein-Jensen (LJ) slants (82 and 76%, respectively, for SDS-NaOH-treated specimens and 78 and 73%, respectively, for NALC-NaOH-treated specimens [8]).

To clarify whether SDS-NaOH would be as suitable as NALC-NaOH, 1,000 clinical specimens were cultivated in the MGIT and BACTEC 460 as well as on LJ and Middlebrook agar biplates (7H10/7H11; for culture conditions, see reference 6). According to the study protocol, PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) was added to all MGITs and to those BACTEC 460 vials which had been inoculated with decontaminated specimens (respiratory, gastric fluid, and urine samples). The statistical significance of differences in recovery rates was determined by the chi² test by using Epi Info (version 6.03; Centers for Disease Control and Prevention, Atlanta, Ga.). The majority of clinical specimens (79.7%) were of respiratory origin (sputum, bronchial aspirate, tracheal aspirate, and bronchoalveolar lavage specimens). The nonrespiratory specimens (20.3%) comprised urine (124 specimens), feces (42 specimens), gastric fluid (21 specimens), and miscellaneous specimens, such as lymph node, cerebrospinal fluid, and pus specimens as well as a large variety of aspirates and biopsy specimens (n = 58).

Nonsterile specimens, i.e., respiratory specimens as well as gastric juice and urine specimens, were pretreated with SDS-NaOH as reported previously (8). Briefly, to 10 ml of a specimen an equal volume of digestant (5% NaOH, 3.16% SDS [Fluka Chemical Company, Buchs, Switzerland]) was added; after being vortexed, the samples were vigorously shaken for 30 min and then left in the rack for another 10 min. Ten milliliters of 1.43% H₃PO₄ (containing 0.006% bromcresol purple as a pH indicator) was added to neutralize the specimen. After a centrifugation step (3,000 × g, 20 min), the pellet was suspended in 10 ml of H₂O and centrifuged again (15 min). The sediment was finally resuspended in 2 ml of 0.067 M phosphate buffer (pH 6.8). Lymph node and tissue specimens were homogenized in a Ten Broek mortar. One hundred microliters of those homogenates as well as 100 μl of other, normally sterile nonrespiratory specimens (cerebrospinal fluid, biopsy samples, pleural aspirates, etc.) was applied to Chocolate II agar (Becton Dickinson) and incubated for 48 h at 36 ± 1°C. Decontamination was carried out only when growth of bacteria or fungi was observed on this medium. Media were inoculated with 0.5 ml (MGIT and BACTEC 460) or 0.25 ml (solid media) of the sediment.

From the 1,000 specimens a total of 92 mycobacterial species were isolated, namely, M. tuberculosis (n = 56), M. avium

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complex (n = 10), M. gordonae (n = 8), M. fortuitum (n = 5), M. kansasii (n = 5), M. szulgai (n = 2), M. xenopi (n = 2), and M. celatum, M. genavense, M. neoaurum, and M. chelonae (n = 1 [each]). Of the nonrespiratory specimens, 11 were positive for AFB: M. tuberculosis was isolated from a lymph node, a pus sample, and two urine specimens; M. avium complex was isolated from feces and two blood samples; M. gordonae was isolated from feces and a gastric fluid specimen; M. xenopi was isolated from gastric fluid; and M. genavense was isolated from liver tissue.

The contamination rates for the MGIT, the BACTEC 460, and solid media were 4.3, 1.6, and 3.8%, respectively. There were 11 specimens containing AFB (8 respiratory samples, 2 feces samples, and 1 gastric fluid specimen) where contamination of one or two media was observed. M. tuberculosis was lost four times due to contamination of the medium (LJ agar [3] and BACTEC 460 [1]), M. avium complex was lost in three cases (LJ medium [2] and MGIT plus Middlebrook agar [1]), and M. fortuitum and M. gordonae were lost in two cases each (MGIT and Middlebrook agar [1] and MGIT [2], respectively).

The MGIT and the BACTEC 460 detected 58.7 and 82.6% of all isolates, respectively, whereas the solid media used in this study detected 56.5% of all isolates (MGIT versus solid media, P > 0.05; MGIT versus BACTEC 460, P < 0.01; BACTEC 460 versus solid media, P < 0.01). For M. tuberculosis the results were similar (64.3% for MGIT, 92.9% for BACTEC 460, and 60.7% for solid media; MGIT versus solid media, P > 0.05; MGIT versus BACTEC 460, P < 0.01; BACTEC 460 versus solid media, P < 0.01) (Table 1). However, only 50% of the NTM were detected by the MGIT and solid media and 66.7% were detected by the BACTEC 460 (P > 0.05 for MGIT versus solid media, MGIT versus BACTEC 460, and BACTEC 460 versus solid media) (Table 1).

The use of a combination of liquid and solid media (current “gold standard” [4]) represents an essential prerequisite for good laboratory practice in mycobacteriology. As expected, when a combination of the MGIT and solid media was compared with a combination of the BACTEC 460 and solid media, there was also a statistically significant difference between these two versions of the gold standard for the recovery of all mycobacteria (67.4 versus 97.8%; P < 0.01) as well as for the recovery of M. tuberculosis (67.9 versus 100%; P < 0.01) and NTM (66.7 versus 94.4%; P < 0.01). The combination of the two liquid media was less efficient in isolating AFB than the BACTEC 460 combined with solid media (87 versus 97.8%; P < 0.01).

The mean time to detection of M. tuberculosis was, on average, significantly longer in the MGIT than in the BACTEC 460 (smear positives, 24.6 versus 13.7 days; smear negatives, 27.4 versus 19.8 days), i.e., nearly comparable to the values obtained on solid media (smear positives, 25.7 days; smear negatives, 32.9 days). The same held true for NTM, for which both the recovery and the average time to detection were, at best, comparable to those obtained on solid media (Table 1).

Overall, no difference between respiratory and nonrespiratory specimens could be found as far as the sensitivity of the MGIT and the average time to detection were concerned. However, since only 20% of the specimens were of nonrespiratory origin, statistical analyses of the recoveries of AFB from respiratory versus nonrespiratory specimens could not be performed.

Generally, there were no problems in reading the MGITs. Reasons for the poor performance of the MGIT after pretreatment of the specimens with SDS-NaOH are most likely associated with the chemical nature of the detergent used for decontamination. Even though sediments underwent several washing steps and their pH was neutral, there may still have been traces of SDS, which is known to strongly bind to the proteins present in the medium. It is thus quite conceivable that the resulting conformational changes taking place in these compounds may severely impair the growth of AFB, leading eventually to both poor recovery and delayed time to detection of mycobacteria.

Our data emphasize that (i) pretreatment with SDS-NaOH is obviously not suitable when using the MGIT for cultivation and (ii) one has to, therefore, strictly adhere to the NALC-NaOH decontamination protocol as recommended by the manufacturer. Palaci et al. (5) pretreated 85 smear-positive specimens by the Petroff method, using NaOH prior to cultivation (2). Although the mean time to detection of M. tuberculosis was about half of that observed in our study (12.6 versus 24.6 days), it is remarkable that the MGIT did not yield results faster than LJ medium. Future studies have to clarify whether NALC-NaOH remains the pretreatment of choice for the MGIT technology since other methods, such as those involving Zephran-trisodium phosphate or oxaic or sulfuric acid (2), may have, for unknown reasons, similar adverse effects.

Becton Dickinson provided us with the MGIT medium.

REFERENCES

TABLE 1. Rates of recovery and times to detection for mycobacteria in clinical specimens (n = 1,000) after pretreatment with SDS-NaOHa

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>M. tuberculosis</th>
<th>NTM (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. detected (%)</td>
<td>No. of days to detection (range)</td>
</tr>
<tr>
<td></td>
<td>All isolates (n = 56)</td>
<td>Smear positive (n = 22)</td>
</tr>
<tr>
<td>MGIT</td>
<td>36 (64.3)</td>
<td>15 (68.2)</td>
</tr>
<tr>
<td>BACTEC 460</td>
<td>52 (92.9)</td>
<td>21 (95.5)</td>
</tr>
<tr>
<td>Solida</td>
<td>34 (60.7)</td>
<td>16 (72.7)</td>
</tr>
</tbody>
</table>

a All values are means.

b LJ medium plus Middlebrook 7H10/7H11 agar (biplate).


