Technique for Extracting Niacin from *Mycobacterium tuberculosis* Cultured on 7H-10 and 7H-11 Agars

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Niacin extractions from *M. tuberculosis* growing on 7H-10 or 7H-11 agar base medium yield consistent results when performed at 37°C for 2 h. The technique does not require any modification of the media formulation.

It is reported that when an agar base medium such as Middlebrook-Cohn 7H-10 (4) or 7H-11 (1) is used for the isolation and identification of *Mycobacterium tuberculosis*, 0.1% l-potassium aspartate (3) or 0.25% l-asparagine (2) should be added to the medium to provide for consistent niacin test results. Unfortunately, the addition of potassium aspartate to the medium negates its use for susceptibility testing, because aspartic acid increases the in vitro minimal inhibitory concentration of isoniazid and streptomycin (3). Also, asparagine, in the recommended concentration, is inhibitory to some strains of *M. tuberculosis* (5). Furthermore, neither of the supplemented media is readily available from commercial sources.

In a limited study (6), it was found that the problem of inconsistent niacin test results was not one of niacin production, but one of niacin extraction. Thus, the study reported here is an extension of that preliminary report to evaluate the modified extraction procedure. This study includes the use of more strains of *M. tuberculosis* plus the addition of 7H-11 medium, as well as the 7H-10 medium as used in the initial study.

The inocula used in this study consisted of a suspension of organisms, which were made by removing portions of several colonies of *M. tuberculosis* growing on Lowenstein-Jensen medium and by emulsifying them in 5 ml of sterile saline. The inocula of the negative control cultures, *M. kansasii*, *M. avium-intracellulare*, and *M. fortuitum*, growing on Lowenstein-Jensen medium were prepared in a similar manner. The 7H-10 and 7H-11 media were inoculated with 0.2 ml of the suspension and incubated in an 8 to 10% concentration of CO₂ at 37°C until there was sufficient growth to test for the presence of niacin. Niacin extraction consisted of flooding the slant cultures with approximately 2 ml of sterile saline and holding the cultures in a near horizontal position for 2 h at 37°C.

A total of 713 isolates of *M. tuberculosis* and 28 negative control cultures growing on 7H-10 and Lowenstein-Jensen medium were tested for the presence of niacin by a method previously described (6), which utilized benzidine-cyanogen bromide. Because of the carcinogenic nature of benzidine, the aniline-cyanogen bromide method (7, 8) was used to test 115 isolates of *M. tuberculosis* and 28 negative control cultures growing on 7H-11 and Lowenstein-Jensen media.

Of the 713 isolates grown on 7H-10, 706 (99.0%) were niacin positive on initial testing by the modified technique. The remaining seven cultures were niacin positive on retest after 1 additional week of incubation. The expected negative niacin test results were obtained from the 50 niacin-negative controls on both the 7H-10 and 7H-11 media (Table 1). There was also an indication that all 115 of the *M. tuberculosis* isolates grown on 7H-11 were niacin positive when tested by the modified technique and that all of the niacin test results reported in this study were confirmed by the standard method (7, 8) when the organisms were grown on Lowenstein-Jensen slants (Table 1).

The consistent niacin test results obtained from *M. tuberculosis* when grown on 7H-10 and 7H-11, using the modified niacin extraction procedure, indicate the reliability of the procedure. Although the modified procedure requires an extraction time of 2 h at 37°C, it has certain advantages over those which require a modification of the media: (i) the prepared agar base media (7H-10 and 7H-11) are readily available from commercial sources; (ii) it can be performed in any laboratory and is not limited to those having a media kitchen; (iii) if media are prepared in-house, the same agar base medium can be used in susceptibility tests in both the
TABLE 1. Results of the modified extraction technique when performed on mycobacteria grown on 7H-10 and 7H-11 media

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. of M. tuberculosis strains</th>
<th>Niacin-negative controls</th>
<th>No. of M. kansasii strains</th>
<th>No. of M. avium-intracellular complex strains</th>
<th>No. of M. fortuitum strains</th>
<th>No. of sterile slant cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Niacin +</td>
<td>Total</td>
<td>Niacin -</td>
<td>Total</td>
<td>Niacin -</td>
</tr>
<tr>
<td>7H-10</td>
<td>713c</td>
<td>706 (99.0%)</td>
<td>10</td>
<td>11</td>
<td>7d</td>
<td>22</td>
</tr>
<tr>
<td>7H-11</td>
<td>115c</td>
<td>115 (100%)</td>
<td>10</td>
<td>11</td>
<td>7</td>
<td>22</td>
</tr>
</tbody>
</table>

a Niacin +, Niacin positive.
b Niacin -, Niacin negative.
c Positive when grown on Lowenstein-Jensen medium and tested by standard technique.
d Positive on retesting after 1 additional week of incubation.

control and drug-containing quadrants; and (iv) the procedure does not influence the growth pattern of the mycobacteria.

LITERATURE CITED