Mechanical Method of Inoculating Plates for Antibiotic Sensitivity Testing

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A mechanical method of inoculating culture plates for antibiotic sensitivity testing is described. This method, which involves the use of a modified laboratory centrifuge, is rapid and provides a homogeneous distribution of organisms.

The single-disc method for antibiotic susceptibility testing of bacteria as described by Bauer et al. (1) is now widely used. Mueller-Hinton agar plates employed in this procedure are inoculated by streaking in three directions with a cotton swab containing the organism to be tested. This communication describes an alternate procedure for inoculation of plates which is rapid and provides a homogeneous bacterial lawn. A related method was described in 1941 by Fennel et al. (2), who used a spring-driven phonograph record turntable to spin agar plates for inoculation of stool suspensions for culture. Recently, Oberhofer and Maddox have mentioned the use of a hand-driven turntable to inoculate antibiotic sensitivity plates (3).

In the method to be described, an inexpensive bench-top centrifuge serves as the basis for a mechanical inoculator. Double-faced cellophane tape is used to attach the cover of a Mueller-Hinton agar plate to the rotor head as shown in Fig. 1. This forms a receptacle for the plates to be inoculated, and the opposing plastic surfaces constitute a friction clutch permitting the plate to be removed and replaced without stopping the rotor. The speed of rotation of the centrifuge is adjusted to 200 to 250 rev/min. (With certain older centrifuge models, an additional rheostat may be necessary to accomplish this.) A Mueller-Hinton agar plate is inoculated by drawing a cotton swab containing the test organisms back and forth once across the diameter of the plate (to obtain an even distribution of organisms). The plate is then placed in its receptacle on the centrifuge. As the plate rotates, the inoculating swab is slowly drawn from the center to the periphery of the plate. A

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**FIG. 1. Modified clinical centrifuge with rheostat showing method of inoculation of plates.**

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**TABLE 1. Representative sample of zone sizes after conventional and mechanical inoculation of culture plates**

<table>
<thead>
<tr>
<th>Organism and antibiotic</th>
<th>Zone size (mm)</th>
<th>Method of inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Conventional</td>
</tr>
<tr>
<td><em>E. coli</em> (ampicillin, 10 μg-disc)</td>
<td>18</td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. aureus</em> (penicillin, 2-unit disc)</td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of zones with respective diameters occurring around five discs on each of two 150-mm Mueller-Hinton agar plates, inoculated with the same bacterial suspension.
yields zones in size with similar testing the plate, we have previously streaking testing, ery for the moved of torsion difference in Table 1). even an the than 1 to 2 mm smaller at the center than at the periphery for the same antibiotic. With the procedure we have described, the inoculation is even and testing with similar antibiotic discs consistently yields zones of inhibition of practically identical size in all positions of the plate.

The centrifuge speed should be kept as close to 200 to 250 rev/min as possible. Microscopic splashing of organisms from the plate due to centrifugal force occurs at speeds of 500 rev/min or greater (but not below 500 rev/min), and above 600 rev/min there is danger of disruption of the agar with gross splattering of the operator and surrounding area.

A simple way to determine if the rotational speed is excessive in the absence of sophisticated equipment is to observe the pattern at the periphery of the plates after incubation. If the speed is correct, a neat, spiral pattern will extend from the center to the periphery of the plate as shown in Fig. 2. However, as the speed is increased, one begins to see radial streaking at the periphery of the inoculated plate (Fig. 3). This starts to occur at speeds of 300 rev/min and above and is a simple warning (with considerable margin of safety before microscopic splashing takes place) that the rotational speed is too high.

Fig. 2. Properly inoculated sensitivity plate. Compact spiral pattern extends from center to periphery.

Fig. 3. Sensitivity plate inoculated at rotational speed of greater than 300 rev/min. Note disruption of spiral pattern at periphery ("radial streaking").

LITERATURE CITED