Capillary Tube Assay for Staphylococcal Enterotoxins A, B, and C

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A miniaturized single-gel diffusion procedure for detection of staphylococcal enterotoxin is proposed. The technique effects substantial savings of reagents and is easy to perform.

Methods of detection of staphylococcal enterotoxins including human volunteers (7), animal tests (3), single-gel diffusion (9), double-gel diffusion (5), slide-Ouchterlony methods (2), fluorescent-antibody (4), hemagglutination-inhibition (6; J. Robinson and F. S. Thatcher, Pasteur Pipette - a Aluminum Cover

Liquid Toxin Sample

Precipitation Band

Antiserum Agar Column

Plasticine Seal

FIG. 1. Capillary tube system for enterotoxin detection.

FIG. 2. Precipitation bands formed in the capillary tubes containing antiserum agar. Concentrations of enterotoxin C in the solutions above the agar were: (A) 25 μg/ml; (B) 50 μg/ml; (C) 100 μg/ml; (D) 200 μg/ml; and (E) 400 μg/ml.

Staphylococcus aureus strains 100, 137, 196E, 217, and S-6; enterotoxins A, B, and C of known concentrations; and specific enterotoxin antisera were obtained through the courtesy of M. S. Bergdoll of The University of Wisconsin, Madison, Wis.

Antiserum was diluted with phosphate-buffered saline (0.02 M, pH 7.4; Weirether et al., 9) and incorporated into a melted, tempered (48 C) 1% agar (Ionagar No. 2, Consolidated Laboratories, Inc., Chicago Heights, Ill.) solution to obtain final dilutions of 1:5, 1:10, and 1:20 of each specific antiserum. By capillary action, about 0.05 ml of the agar-antiserum mixture was brought to approximately one half the length of the thinner portion of a Pasteur pipette (9 inches in size). The tip of the Pasteur pipette was sealed with Plasticine. To each capillary tube containing specific antiserum agar, 0.1 ml of a specific enterotoxin or test sample was added; a quick jerk of the Pasteur pipette will cause the liquid sample to come in contact with the antiserum agar in the capillary tube. The concentra-
tions added ranged from 1.25 to 400 μg/ml to establish standard enterotoxin curves. Culture supernatants for testing were obtained by centrifugation of 24-hr broth cultures (37°C) of S. aureus strains grown on a rotary shaker in the following medium: 3% protein hydrolysate (Mead Johnson International, Evansville, Ind.), 3% NZ-amine NAK (Sheffield Chemical, Norwich, N.Y.), and 0.5 μg/ml each of niacin, thiamine, and pantothenate. Final pH values of the medium after growth ranged from 7.2 to 7.5. Pasteur pipettes containing toxin samples overlying the antiserum agar in the capillary portions were covered with aluminum foil and incubated in a moist chamber at 37°C for 10 to 24 hr (Fig. 1).

After incubation, the length of the zone of precipitation in the antiserum agar, indicating the presence of the specific toxin, was measured in millimeters (Fig. 2). Standard toxin curves plotting concentration (micrograms per milliliter) against band length (millimeters) were made on semi-log paper for enterotoxins A, B, and C (Fig. 3). Although standard curves for other antiserum dilutions of all three toxins were established, Fig. 3 only presents those dilutions that provided clearest band observations.

![Fig. 3. Standard enterotoxin curves. Symbols: Δ, enterotoxin A; O, enterotoxin B; and □, enterotoxin C. Dilutions for antisera A, B, and C were 1:5, 1:10, and 1:10, respectively. Results are average of at least six tests.](http://aem.asm.org/)

### Table 1. Comparison of enterotoxin detection by two methods

<table>
<thead>
<tr>
<th>Strains</th>
<th>Capillary tube method</th>
<th>Modified hemagglutination-inhibition method</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus 100</td>
<td>2.5 (A)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S. aureus 196E</td>
<td>1.6 (A)</td>
<td>4.68 (A)</td>
</tr>
<tr>
<td>S. aureus S-6</td>
<td>200.0 (B)</td>
<td>200–250 (B)</td>
</tr>
<tr>
<td>S. aureus 137</td>
<td>3.0 (C)</td>
<td>7.02 (C)</td>
</tr>
<tr>
<td>S. aureus 217</td>
<td>2.7 (C)</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Amounts of toxins in micrograms per milliliter of culture supernatant and types of toxins detected. Results are average of at least six tests.

<sup>b</sup> Not determined.

Two straight-line slopes were obtained for each toxin measured: one slope for toxin concentration greater than 10 μg/ml and the other for toxin concentrations smaller than 10 μg/ml. Capillary action may account for this phenomenon. Precipitation band lengths of unknown samples could then be matched with these curves. Concentrations and kinds of enterotoxins present could then be estimated. The lower threshold of sensitivity is ca. 1 to 2 μg of toxin per ml of liquid medium. The type and quantity of enterotoxin in culture supernatants of several enterotoxic S. aureus strains detected by the capillary tube method and a modified hemagglutination-inhibition procedure (D. Y. C. Fung, Ph.D. Thesis, Iowa State University, Ames, 1969) are listed in Table 1. Comparable results were obtained in testing toxin production of S. aureus strains 196E, S-6, and 137 by the two methods. Practical applications including toxin detection in soybean milk and culture supernatants of irradiated S. aureus were successfully achieved by the capillary tube method.

The capillary tube method thus offers a miniaturized and reliable procedure for staphylococcal enterotoxin detection in culture supernatants as well as in extracts of food.

### LITERATURE CITED


