Serotyping of Noncapsular *Haemophilus influenzae*

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A microtechnique is described for agglutination typing of *Haemophilus influenzae*. It also provides a further means for classification and study of noncapsular type-specific strains.

This paper is concerned with a study of the serotyping of *Haemophilus influenzae* by using the agglutination microtechnique described by Kirkman et al. (1).

Relatively high agglutination titers were obtained for some *H. influenzae* strains typed by this microtechnique, suggesting the presence of non-type-specific capsular agglutinins in the commercial typing sera used. This possibility was further strengthened by the fact that *H. influenzae* isolated from nonhospitalized patients could be typed by a 1:200 serum dilution (at 64 to 1,024 button units), whereas strains from hospitalized patients required the use of a 1:20 serum dilution (4 to 8 button units). In enriched broth, respiratory strains consistently produced distinct turbidity with sediment in contrast to the turbid growth without sediment produced by strains isolated from infected body fluids. A similar observation was made by Pittman and Davis (2) with regard to nontypeable and typeable strains.

Antigens used for typing consisted of young formalinized cultures grown in Trypticase Soy Broth (BBL) enriched with 1% supplement C for a source of X and V factors (Difco) and 1% fresh frozen normal rabbit serum (Robbin Laboratories, Inc., Chapel Hill, N.C.). Buffer for the microtechnique was formalinized phosphate-buffered saline (pH 7.2) containing 1% fresh frozen normal rabbit serum. The organisms were stained by the use of a 0.001% solution of methylene blue in phosphate-buffered saline (pH 7.2). Tests were performed in V bottom Microtiter plates (no. 220-25, Cooke Engineering Co., Alexandria, Va.) by using test volumes of 0.025 ml incubated in a moist chamber at 20 and 4 C. Tests were read against a white background after the plates were centrifuged for 30 min at 5 to 6 X g.

Fig. 1. *Haemophilus influenzae* type b, a nasopharyngeal strain isolated from the hospitalized patient. By using a Microtiter plate, two to four button units of antigen were tested in serial twofold dilutions of type-specific antisera a-f (Burroughs-Wellcome). One button unit of antigen is the highest twofold dilution of a young formalinized broth culture that will produce a grossly visible distinct button of stained bacteria at the bottom of the well. Grossly visible agglutination was observed in only type b serum diluted 1:2 through 1:64.

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serotypes isolated from hospitalized patients produced homologous serum agglutination titers of either 1:64 or 1:128, whereas *H. influenzae* isolated from out-patients showed homologous serum agglutination titers that ranged from 1:2,048 to 1:32,768. This difference in serum titers is felt to be due to two different types of agglutinins being detected: (i) the capsular type-specific agglutinin; (ii) the predominating non-capsular somatic agglutinin.

The results shown in Table 1 indicate that all strains react only with one of the six antisera and the majority of strains from out-patients reacted with serum for type c. The probability that all sera possessed both capsular specific and nonspecific somatic antibody was suggested by the manufacturer in that all sera are made by using encapsulated types for immunizing the rabbits. The range of titers (Table 1) indicates that the microtechnique is capable of detecting agglutinins in extreme dilution and should offer a means for further classification and study of nonencapsulated respiratory *H. influenzae* strains.

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**Notes**
- Nasopharynx specimens from out-patients were obtained from Frank Loda, Frank Porter Graham Child Development Center, University of North Carolina, Chapel Hill. *H. influenzae* strains isolated by the hospital laboratory staff from hospitalized patients were supplied by Janet Fischer, Department of Medicine, University of North Carolina, Chapel Hill.

**LITERATURE CITED**

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**Table 1. Serotyping of *Haemophilus influenzae***

<table>
<thead>
<tr>
<th>Source</th>
<th>Patient category</th>
<th>No. of patients</th>
<th>No. of <em>H. influenzae</em></th>
<th>Distribution of type</th>
<th>Homologous serum agglutination titers (number of strains/type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharynx</td>
<td>Out-patients</td>
<td>35</td>
<td>80</td>
<td>0 0 1 0 37 6</td>
<td>1:64 1:128 1:2,048 1:32,768 4/a 37c,6/d 1/f</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>In-patients</td>
<td>5</td>
<td>5</td>
<td>1 2 0 0 0 0</td>
<td>1/a 1/b 2/c 2/b</td>
</tr>
<tr>
<td>Trachea</td>
<td>In-patients</td>
<td>1</td>
<td>1</td>
<td>0 0 0 0 0 0</td>
<td>1/a 1/b 2/c 2/b</td>
</tr>
<tr>
<td>Sputum</td>
<td>In-patients</td>
<td>5</td>
<td>5</td>
<td>0 4 0 0 0 0</td>
<td>1/a 1/b 2/c 2/b</td>
</tr>
<tr>
<td>Blood</td>
<td>In-patients</td>
<td>4</td>
<td>4</td>
<td>0 2 0 0 0 0</td>
<td>1/d 2/b</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>In-patients</td>
<td>3</td>
<td>3</td>
<td>0 2 0 0 0 0</td>
<td>1/d 2/b</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>53</td>
<td>98</td>
<td>6 12 40 7 0 1 32</td>
<td>11 7 4 44</td>
</tr>
</tbody>
</table>

*a* Burroughs-Wellcome sera.

*b* Also tested in the quellung reaction with Difco sera.