Evaluation of the Phadebact and Bactigen Reagents for Detection of *Neisseria meningitidis* in Cerebrospinal Fluid

TERRENCE A. KURZYNSKI,* JUDITH L. KIMBALL, MARCIA B. POLYAK, GEORGE S. CEMBROWSKI,† AND RONALD F. SCHELL

*State Laboratory of Hygiene, University of Wisconsin, Madison, Wisconsin 53706*

Received 13 November 1984/Accepted 21 February 1985

The Phadebact and Bactigen reagents were evaluated for detection of *Neisseria meningitidis* in cerebrospinal fluids. The Bactigen test yielded stronger agglutination reactions from clinical specimens and was significantly more sensitive when used with whole-cell suspensions and purified antigens.

Diagnosis of meningococcal meningitis, although aided occasionally by Gram stain results, is usually based on clinical presentation. Rapid test such as Gram stain, counterimmunoelectrophoresis, latex particle agglutination, coagglutination, and enzyme-linked immunosassay have been used with limited success for rapid detection of bacterial antigens (1, 2, 4−9). The incidence and severity of meningococcal meningitis have prompted the development of rapid diagnostic methods. For this purpose, we evaluated the Phadebact and Bactigen reagents, designed specifically to guide therapy by rapid detection of *Neisseria meningitidis* in cerebrospinal fluids (CSF).

The Phadebact coagglutination test (Pharmacia Diagnostics, Piscataway, N.J.) and the Bactigen latex agglutination test (Wampole Laboratories, Cranbury, N.J.) for *N. meningitidis* were performed by the procedure recommended by the manufacturer. The Bactigen test contains separate antibodies for the detection of *N. meningitidis* groups A, B, C, and Y, whereas the Phadebact reagent is polyvalent for groups A, B, C, Y, and W135. The results of the agglutination reactions for both tests were graded 0 (no reaction), 1+ (barely visible fine agglutination), 2+ (small clumps against a cloudy background), 3+ (medium-sized clumps against a cloudy background), and 4+ (large clumps against a clear background). Reactions with the Phadebact test were examined at 1-min intervals for 4 min, whereas the Bactigen test was read after 10 min.

Ninety-five CSF specimens were obtained from hospitals throughout Wisconsin. Specimens were frozen at −20°C and mailed to the State Laboratory of Hygiene where they were stored at −60°C until used. *N. meningitidis* was cultured from 18 CSF specimens, and then the bacteria were grouped with reference sera obtained from the Centers for Disease Control (Atlanta, Ga.). They included 11 organisms from group B, 4 from group C, and 1 from group W135. The group of the remaining two meningococcal isolates was not determined. *Haemophilus influenzae* was isolated from 24 CSF specimens, and 15 of these isolates were type b. An additional culture-negative specimen was positive for *H. influenzae* type b by counterimmunoelectrophoresis. Six CSF samples were from patients with *Streptococcus pneumoniae* meningitis. Two additional specimens were negative for growth but were positive for *S. pneumoniae* by counterimmunoelectrophoresis.

### TABLE 1. Number of type of CSF specimens tested with the Phadebact and Bactigen reagents for *N. meningitidis*

<table>
<thead>
<tr>
<th>Commercial reagent</th>
<th>No. and type of bacterial isolates in CSF</th>
<th>No. of control CSF specimens</th>
<th>No. of CSF specimens tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>N. meningitidis</em></td>
<td><em>H. influenzae</em></td>
<td><em>S. pneumoniae</em></td>
</tr>
<tr>
<td>Phadebact</td>
<td>18</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>Bactigen</td>
<td>14</td>
<td>21</td>
<td>NT</td>
</tr>
</tbody>
</table>

a One specimen was culture negative for *H. influenzae* but positive by counterimmunoelectrophoresis.

b Two specimens were culture negative for *S. pneumoniae* but positive by counterimmunoelectrophoresis.

c Control CSF were negative by culture and Gram stain.

d NT, Not tested.

munoelectrophoresis. Nineteen of the specimens had the following organisms isolated from the CSF: *Listeria monocytogenes* (five specimens), coagulase-negative *Staphylococcus* spp. (four specimens), *Staphylococcus aureus* (four specimens), *Escherichia coli* (three specimens), and one specimen each with *Streptococcus* spp., *Cryptococcus* spp., and *Streptococcus* spp. with coagulase-negative *Staphylococcus* sp. There were also 25 specimens negative by culture and Gram stain. Ninety-five of these specimens were tested with Phadebact, and forty-nine were tested with Bactigen (Table 1).

The sensitivity of the Phadebact and Bactigen reagents was also evaluated by using dilutions of meningococcal whole-cell suspensions and purified antigens. *N. meningitidis* groups A, B, and C were grown on chocolate agar, suspended in saline, and adjusted to 10⁶ organisms per ml with a McFarland 0.5 standard. Tenfold dilutions were made, boiled for 5 min, and cooled before testing. Purified antigens were obtained from the Bureau of Biologics, Public Health Service, Atlanta, Ga.

* Corresponding author.

† Present address: Pepper Laboratory, Hospital of the University of Pennsylvania, Philadelphia, PA 19104.
Health Service, Bethesda, Md., and diluted in saline. All antigens were stored at −60°C until used.

The sensitivity (3; true-positive rate) of the Phadebact test for *N. meningitidis* was 78% when the reactions were read after 1 min (data not shown) and 78% after 4 min (Table 2). All reactions, however, were weak (11 were 1+ and 3 were 2+). When the data were analyzed according to serogroups, the sensitivities after 1 min were 36% for group B (4 of 11), 0% for group C (0 of 4), 0% for group W135 (0 of 1), and 50% (1 of 2) for those not grouped. These values increased to 73% (8 of 11), 75% (3 of 4), 100% (1 of 1), and 100% (2 of 2), respectively, after 4 min, with an average sensitivity of 78%. The sensitivity of the Bactigen test was also 78%. The Bactigen test detected 82% (9 of 11) of group B and 67% (2 of 3) of group C. However, Bactigen reactions were easier to interpret (≥3+ reactions), and there were no cross-reactions.

The specificity (3) of the Phadebact test was 88% (Table 2). Of 25 specimens, 9 (36%) that were positive by culture or counterimmunoelectrophoresis for *H. influenzae* gave a 1+ reaction with the Phadebact reagent for detection of *N. meningitidis* within 2 to 4 min. Specificity increased to 99% when these CSF specimens were examined concurrently with the Phadebact *H. influenzae* reagent, since homologous reactions were stronger (4+). No false-positive reactions were detected in CSF specimens positive for *S. pneumoniae* or the 25 control specimens. In addition, no false positives were detected with CSF containing other pathogens. The specificity of the Bactigen test was 100%, although false-positive reactions were not determined in CSF containing *S. pneumoniae* and *H. influenzae*.

The sensitivity of the Phadebact test for detecting purified antigens A and C was 10^4 to 10^2 ng/ml (Table 3). These reactions were weak (±2+) and occurred only within a narrow range of antigen concentrations. The Phadebact test also failed to agglutinate all concentrations of purified group B antigen. In contrast, the Bactigen test detected a wide range of purified antigens with a strong reaction. Only the Bactigen group B reagent failed to detect antigen below 1 ng/ml. When boiled suspensions of individual cultures of meningococcal groups A, B, and C were used to determine sensitivity, the Bactigen test detected 10^3 organisms per ml with reagents A and B and 10^2 organisms per ml with reagent C. The Phadebact test detected 10^3 group A, 10^2 group B, and 10^1 group C organisms per ml. These results show that the Bactigen test was ca. 10,000-fold more sensitive than the Phadebact test.

Although the sensitivity of the Phadebact test was 78%, most reactions were weak (1+) and open to interpretation. Similar results were obtained by Tilton et al. (7). Many of these reactions would be overlooked in laboratories where few positive CSF specimens are obtained. The Phadebact test also produced false-positive reactions in CSF containing *H. influenzae*. Therefore, the Bactigen test has advantages since it yielded strong reactions and was more sensitive for detecting small quantities of purified antigens and whole cells. The specificity of the Bactigen test was 100%, but CSF containing *S. pneumoniae* and *H. influenzae* were not examined.

In conclusion, the Bactigen test was more sensitive than the Phadebact test. Since direct Gram stains and cultures of CSF specimens have limitations (2, 5, 6, 10), it may be advantageous to supplement these procedures with the Bactigen test.

This study was supported in part by a grant from Pharmacia, Inc., Piscataway, N.J.

We thank microbiologists from hospitals in Wisconsin for providing the CSF specimens. Donna Moffet, Microbiology Supervisor at Madison General Hospital, was especially cooperative.

**LITERATURE CITED**


