Comparison of Phadebact Coagglutination, Bactogen Latex Agglutination, and Counterimmunoelectrophoresis for Detection of Haemophilus influenzae Type b Antigens in Cerebrospinal Fluid

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Cerebrospinal fluid specimens from patients with suspected meningitis were screened with the Phadebact Haemophilus Test (Pharmacia Diagnostics), with Bactogen (Wampole Laboratories), and by counterimmunoelectrophoresis. With culture-positive fluids, Phadebact coagglutination detected 95%, Bactogen latex agglutination detected 91%, and counterimmunoelectrophoresis detected only 79%. Both agglutination techniques were 25-fold more sensitive than counterimmunoelectrophoresis when tested with dilutions of positive fluids. To obtain specific reactions with the Phadebact reagents it was necessary to heat treat (95°C, 5 min) the fluid; with Bactogen and counterimmunoelectrophoresis this was not necessary.

Detection of bacterial antigens in cerebrospinal fluid (CSF) has enabled the rapid identification of etiological agents causing meningitis (6–8, 14, 15). Rapid identification allows the appropriate choice of chemotherapy at an early stage and can provide prognostic information based on quantitation of the antigen (4). In cases in which cultures are sterile because only low numbers of viable organisms are present in CSF or because of previous antibiotic therapy, antigen detection permits the identification of the infective agent. Techniques used to detect antigens in CSF have included counterimmunoelectrophoresis (CIE) (2, 3, 14), latex particle agglutination (6, 8, 13, 15), coagglutination (1, 9), and enzyme-linked immunosorbent assay (16). CIE has been the most widely used method of detecting antigens, although it requires specialized equipment, takes a relatively long time to perform (30 to 60 min), and fails to detect antigen in substantial numbers of patients (11, 12, 14). Latex agglutination has proven to be a highly sensitive test for detecting Haemophilus influenzae type b capsular antigen in CSF specimens (6, 8, 12, 13), but it has been unavailable in commercial diagnostic kits until recently. Coagglutination has also been found to be a rapid, sensitive test for the detection of H. influenzae type b (5). A commercial diagnostic kit (Phadebact Haemophilus Test; Pharmacia Diagnostics, Piscataway, N.J.) utilizing the coagglutination principle is available for identification of isolates and antigen but has not been evaluated with CSF specimens obtained from patients with suspected meningitis. The present study compares three methods for their sensitivity and specificity in detecting H. influenzae type b antigen. CIE, Phadebact coagglutination, and Bactogen (Wampole Laboratories, Cranbury, N.J.) latex agglutination were compared with CSF specimens obtained from patients with suspected meningitis.

MATERIALS AND METHODS

Specimens. CSF specimens from children with meningitis were selected for analysis if they were culture positive for H. influenzae or if they came from patients with previous culture-positive CSF specimens. Culture-negative CSF specimens were selected at random after being submitted to the laboratory and shown to be culture negative. Specimens that were culture positive for other organisms were selected for specificity tests. These specimens were from cases with evidence of clinical infection. Fluids were stored at 4°C until culture results were obtained and then analyzed for antigens immediately, or were frozen at −20°C and tested later.

CIE. CIE was performed by a standard method (2) with antiserum to H. influenzae type b obtained from Statens Serum Institute (Copenhagen, Denmark) and from Hyland Diagnostics (Deerfield, Ill). Both antisera gave identical results.

Coagglutination. Phadebact Haemophilus coagglutination was performed according to the manufacturer’s instructions and rated on a scale of 1 to 4+ after 2 min of rocking on a glass slide. Two reagents, a type b reagent and a reagent inclusive for types a and c through f, were tested on each fluid. Any agglutination...
rated 1+ or greater was considered positive. CSF specimens were tested directly, but in most cases this was found to give nonspecific agglutination, so fluids were also heat treated at 95°C for 5 min before testing.

**Latex agglutination.** Bactogen latex agglutination was also performed as recommended by the manufacturer. Dilutions of CSF used in the tests were made with buffer supplied for this purpose with the Bactogen kit. Tests with this kit consisted of performing agglutination with both a positive and negative antigen control and with a negative reagent control for each test run. Agglutination was carried out for 10 min on a rotary shaker at 140 rpm, and the reactions were scored as positive or negative. Fluids were tested both before and after heat treatment.

**RESULTS**

Of 21 CSF specimens which were culture positive for *H. influenzae* type b, coagglutination detected antigen in 20, latex agglutination detected antigen in 19, and CIE detected antigen in 15 (Table 1). Of these positive fluids, Gram staining was performed on 19 and found to be positive for 15. In the one case in which coagglutination was negative, Gram staining, latex agglutination, and CIE were also negative, despite recovery of *H. influenzae* type b by culture. Follow-up culture-negative CSF specimens from two cases previously shown by culture to be due to *H. influenzae* type b gave positive coagglutination results; however, latex agglutination and CIE were negative with fluid from one of these cases (Table 1).

To test the sensitivity of each method, serial fivefold dilutions of 19 *H. influenzae* type b positive CSF specimens were made, and each dilution was tested by each antigen detection method. Both coagglutination and latex agglutination were found to be approximately 25-fold more sensitive than CIE since fluids diluted by at least that much more after negative CIE tests still gave positive reactions in the agglutination tests (Fig. 1). Latex agglutination appeared to be slightly more sensitive since 15 to 20% more of the CSF specimens were positive by latex agglutination than by coagglutination at dilutions of 1/625 and 1/3,125.

Both coagglutination and latex agglutination were highly specific. When tested against a variety of culture-positive specimens containing agents other than *H. influenzae*, no agglutination reactions were observed. The culture-positive fluids tested included: *Neisseria meningitidis*, 5; *Streptococcus pneumoniae*, 7; *Staphylococcus epidermidis*, 3; and one each of *Neisseria lactamica*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter cloacae*, and *Candida tropicalis*. No other agglutination reactions were observed with 50 additional culture-negative fluids. CIE showed the same high level of specificity.

To obtain these specific results with the Phadebact reagents, it was necessary to heat treat (95°C, 5 min) the specimens before testing. Without heat treatment, coagglutination was highly nonspecific in that the reagents both for *H. influenzae* type b and for types a and c through f reacted with many positive CSF specimens (Table 2). With Bactogen, heat treatment was not found to be necessary with any specimen.

**DISCUSSION**

These results demonstrate a distinct advantage of rapid agglutination tests over CIE in detecting *H. influenzae* type b antigen in CSF.
TABLE 2. Effect of heat treating CSF specimens on the specificity of antigen detection results

<table>
<thead>
<tr>
<th>Organism isolated</th>
<th>No. of specimens</th>
<th>Agglutination</th>
<th>Heat treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No heat treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phadebact test with reagents:</td>
<td>Bactogen test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type b</td>
<td>Types a, c-f</td>
</tr>
<tr>
<td>H. influenzae type b</td>
<td>13</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N. lactamica</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. freundii</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Both coagglutination and latex agglutination were more sensitive and highly specific as performed with the reagents supplied in the two diagnostic kits. Both were also more sensitive than Gram staining. The increased sensitivity over CIE found with latex agglutination has been demonstrated not only with CSF specimens but with serum and urine specimens from patients with bacteremia and other H. influenzae type b infections (6, 12, 14). Previous evaluations showing the increased sensitivity over CIE have been carried out both with laboratory-prepared reagents and with Bactogen (13). Latex agglutination has been shown to detect 0.2 to 0.5 ng of purified polyribosylphosphate capsular antigen per ml (11, 12), whereas CIE detected 5 to 10 ng of antigen per ml (12). Since previous studies have shown that significant numbers of patients have antigen levels lower than 10 ng/ml (10), this increased sensitivity is very relevant diagnostically. The availability of this sensitivity in the Bactogen kit should enhance the use of this diagnostic approach.

Although latex agglutination was found to be slightly more sensitive than coagglutination, additional specimens would have to be compared to determine whether this was a significant finding. Both tests significantly enhanced detection over CIE. The one disadvantage of coagglutination was its nonspecific nature if the specimens were not heat treated. This has been found previously with CSF specimens containing high levels of protein or blood but is easily overcome by pre-treating the CSF with protein A (9) or by briefly heating the fluids (14). Heat treatment of the specimens examined here still allowed completion of the test within a short period of time and completely abolished nonspecific reactions.

Previous comparisons of coagglutination to latex agglutination for detecting H. influenzae type b antigen in CSF have yielded varied results. Coagglutination has been found to be both less sensitive (1) and of equal sensitivity (14). Since previous studies have been carried out with laboratory reagents prepared with different antisera and with protein A containing staphylococci, it is difficult to evaluate the results. The present report, comparing two commercially available kits and CIE with the same specimens indicates, that both agglutination techniques can achieve similar levels of sensitivity.

The availability of these two diagnostic kits, which are easy to use, require only limited materials, and take only a few minutes to perform, should advance the rapid diagnosis of H. influenzae type b meningitis.

LITERATURE CITED

7. Leinonen, M., and H. Kayhty. 1978. Comparison of counter-current immunoelectrophoresis, latex agglutination, and radioimmunoassay in detection of soluble capsu-
lar polysaccharide antigens of *Haemophilus influenzae* type b and *Neisseria meningitidis* of groups A or C. J. Clin. Pathol. 31:1172-1176.