Identification of *Streptococcus pneumoniae* by the Phadebact Coagglutination Test

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The Phadebact Pneumococcus Test is a coagglutination slide test for the serological identification of *Streptococcus pneumoniae*. Of 200 alpha-hemolytic streptococcal isolates, coagglutination test results agreed with those of optochin susceptibility and bile solubility in 189 cases, 105 of which were identified as *S. pneumoniae* by all three methods. The Phadebact test was 100% (113 of 113) sensitive and 98% (85 of 87) specific and was more sensitive than counterimmunoelectrophoresis in detecting the presence of pneumococcal antigen in cerebrospinal fluid. In fluids seeded with known amounts of pneumococcal antigen, it consistently detected lower levels than did counterimmunoelectrophoresis. The test provides a rapid and simple method for the definitive identification of *S. pneumoniae*.

*Streptococcus pneumoniae* is the most common cause of bacterial pneumonia and the second most frequent cause of bacterial meningitis (20); it has been the cause of increasing concern because of recent reports of isolates relatively resistant to penicillin (1, 13, 22). Until recently, the only definitive method available for identifying *S. pneumoniae* was the Quellung capsular precipitin reaction, using specific antiserum to the capsular polysaccharide (18). This method is quite specific but requires the availability of antisera of high titers and a certain amount of expertise to produce accurate results. Because of these factors, the Quellung test is not routinely performed in many clinical laboratories. The most popular method for differentiating *S. pneumoniae* from other alpha-hemolytic streptococci is optochin susceptibility, a presumptive method which requires overnight incubation of the isolated organism (4). Although this test is widely used, there are many variations of the procedure, involving incubation atmosphere, disk size, and zone of inhibition size. R. Facklam (11) has suggested several steps to standardize the procedure, including an optochin zone size which designates isolates as possible pneumococci. These questionable isolates should then be tested by bile solubility, another presumptive test. The bile solubility test recommended by Facklam involves overnight incubation in broth, neutralization with NaOH, and addition of sodium deoxycholate followed by a 2-h incubation period. Although Facklam reports bile solubility alone to be 86% sensitive, it provides a higher accuracy rate in combination with optochin susceptibility, but is a more involved procedure and lengthens the time for identification up to 72 h.

Within the past several years other rapid immunological techniques have been applied to pneumococcal identification, including counterimmunoelectrophoresis (CIE), enzyme-linked immunosorbent assay, and agglutination methods (7, 12, 15). In 1973, Kronvall (15) reported on a slide coagglutination test for typing *S. pneumoniae*. This method utilizes staphylococci sensitized with Omni-sera (Statens Seruminstitut, Copenhagen, Denmark) containing antibodies to all 83 known pneumococcal capsular, polysaccharide type antigens. This method was further utilized by Edwards et al. (8, 9) to test for pneumococcal antigens in sputum and serum. Recently, Pharmacia Diagnostics, Piscataway, N.J., has released a test kit, Phadebact Pneumococcus Test, for the identification of *S. pneumoniae* isolates by coagglutination. The purpose of this study was to compare the Phadebact Test with optochin susceptibility and bile solubility. The ability of the Phadebact system to directly detect pneumococcal antigen in cerebrospinal fluid (CSF) was also investigated, as well as the minimum level of purified pneumococcal capsular polysaccharide detectable by Phadebact and CIE.

**MATERIALS AND METHODS**

**Organisms.** Two hundred alpha-hemolytic streptococcal isolates with typical pneumococcal morphology were used. Optochin susceptibility, bile solubility, and coagglutination testing were performed on the same fresh subcultures, which had been streaked onto sheep blood agar plates and incubated in 5% CO₂ for 18 to 24 h. Isolates with a discrepancy for any of the three methods were tested with Statens Seruminstitut Omni-
Omni-sera were biochemically sera. Those which were negative with Omni-sera were biochemically identified (10). Isolates positive with Omni-sera were tested with Statens Seruminstitut anti-sera to individual serotypes 1, 3, 4, 6, 7, 14, 18, 19, and 23. These serotypes were chosen because of their frequency of isolation in the United States (6). Serotyping was done by CIE, using the method of Sottile and Rytel (24). Since serotypes 7 and 14 do not migrate toward the anode without a specially prepared buffer system (2, 14), these two antigens were tested for by immunodiffusion (6).

To perform optochin susceptibility, a 6-mm optochin disk (BBL Microbiology Systems, Cockeysville, Md.) was placed on a sheep blood agar plate streaked with several colonies. The plate was incubated for 18 to 24 h in 5% CO₂ at 35°C. A zone of inhibition of ≥14 mm was considered positive. A light suspension of the organism was made in two tubes, each containing 0.5 ml of buffered broth, for the bile solubility test. Three drops of 10% sodium deoxycholate were added to one tube, and 3 drops of physiological saline were added to the other as a control. After 15 min, any clearing in the tube with deoxycholate as compared with the control was considered positive (19). For the Phadebact Pneumococcus Test a clean, dry microscope slide was divided into a test side and a control side. One drop of pneumococcal reagent (a mixture of specific pneumococcal antibodies raised in rabbits to all of the polysaccharide capsular antigens of S. pneumoniae strains) was placed on the test side, and 1 drop of control reagent (gamma globulin from nonimmunized rabbits coupled with protein A) was placed on the control side. With a loop, a smear of the culture containing three to five colonies was made next to each drop and then thoroughly mixed with the corresponding reagent, using an applicator stick. The slide was rocked for up to 2 min and observed, using indirect lighting against a dark background, for the formation of a coagglutination lattice significantly stronger than the control.

CSF. Thirty CSFs previously cultured and tested for the presence of S. pneumoniae capsular antigen by CIE were directly tested by the Phadebact Pneumococcus Test. Each CSF was inoculated onto chocolate and blood agar plates and incubated in 5% CO₂ at 35°C for up to 4 days before being considered negative for bacterial growth. S. pneumoniae cells cultured from positive spinal fluids were identified by optochin susceptibility and bile solubility. To perform CIE, a Hyland (Hyland Diagnostics, Deerfield, Ill.) electrophoresis power supply and CIE supply package were used. CSF samples were electrophoresed at 30 mA for 60 min against Statens Seruminstitut S. pneumoniae Omni-sera and observed for precipitin lines, using a Hyland Immuno-Illuminator. For the Phadebact Pneumococcus Test, 1 drop of CSF was mixed with 1 drop of pneumococcal reagent and observed for reactivity compared with the negative control.

Pneumococcal capsular polysaccharide. Purified pneumococcal capsular polysaccharide in 1,000-μg amounts from serotypes 1, 3, 6A, 7, 8, and 14 (donated by Lederle Laboratories, Pearl River, N.Y.) was dissolved and diluted in phosphate-buffered saline. The following amounts were used to compare the sensitivity of CIE and Phadebact, following the same procedure as for CSF: 100, 10, and 1 μg/ml, and 100, 50, 25, 10, 5, and 1 ng/ml.

**RESULTS**

Of the 200 alpha-hemolytic streptococcal isolates, coagglutination test results agreed with those of optochin susceptibility and bile solubility in 189 cases. Of these, 105 were identified as S. pneumoniae by all three tests, whereas 84 were negative by all three methods. The 11 isolates in which discrepancies occurred were further tested (Table 1). Of these, eight were identified as S. pneumoniae by the Omni-sera,
and six of these were positive by the Phadebact and bile tests but were optochin resistant. The other two were Phadebact positive and optochin susceptible but were not soluble in bile. Of the three remaining isolates not reactive with the Omni-sera, two were Phadebact positive and resistant to optochin, but were not soluble in bile, and one was optochin susceptible but Phadebact and bile negative. These last three isolates were subsequently biochemically identified as *Streptococcus sanguis*, *Streptococcus mitis*, and *S. sanguis*, respectively.

Results of testing CSFs for the presence of pneumococcal polysaccharide are shown in Table 2. The Phadebact Test detected 7 of 10 CSFs culture positive for *S. pneumoniae*, whereas CIE detected 4. None of the CSFs containing nonpneumococcal organisms exhibited cross-reactivity with the coagglutination antisera. The levels of pneumococcal capsular polysaccharide which were detected for several serotypes of *S. pneumoniae* by CIE or immunodiffusion and Phadebact are listed in Table 3. Phadebact consistently detected lower levels of pneumococcal polysaccharide than did CIE. With the use of immunodiffusion, serogroups 7 and 14 were detectable at 10,000 ng/ml, whereas Phadebact was positive to levels of 50 ng/ml.

**DISCUSSION**

Pharmacia coagglutinating reagents for the identification of beta-hemolytic streptococci groups A, B, C, and G, group D streptococci, *Neisseria gonorrhoeae*, and *Haemophilus influenzae* type b are currently available and have been favorably evaluated (16, 17, 21, 23; N. M. Burdash, R. T. Newell, and M. E. West, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, C94, p. 278). Comparison of these coagglutination tests with standard methodologies have yielded agreement rates for specificity and sensitivity ranging from 97.9 to 100%. In the present study, based on the isolates in agreement by all three methods plus the eight isolates identified by the Omni-sera (Table 1), the Phadebact test correctly identified all pneumococcal isolates for a sensitivity rate of 100% (113 of 113), whereas only two of the nonpneumococcal alpha-hemolytic streptococci gave a positive reaction, for a specificity rate of 98% (85 of 87). The bile solubility test was 98% sensitive and 100% specific, and the optochin susceptibility test was 95% sensitive and 99% specific. If Facklam's advice is followed and any isolate with an optochin zone of inhibition between 6 and 14 mm is tested for bile solubility, four of the six isolates would then have been correctly identified as *S. pneumoniae*. It thus appears that coagglutination, bile solubility, and optochin combined with bile solubility are comparable with regard to identification of *S. pneumoniae*.

The coagglutination procedure does provide several advantages over the physiological tests. Slide agglutination procedures are generally eagerly accepted in the laboratory because of their simplicity. No specialized equipment or training is needed, so this test can also be performed in any clinical laboratory capable of growing the organisms. Identification is definitive in that serological reactivity is used as opposed to presumptive identification by biochemical testing or antimicrobial susceptibility. The importance of rapid and definitive identification cannot be overstated with regard to systemic infections such as meningitis, septicemia, and pneumonia in that time is of the essence in the identification

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**TABLE 2. Detection of *S. pneumoniae* capsular polysaccharide antigen in CSF by Phadebact and CIE**

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of CSFs</th>
<th>No. Phadebact positive</th>
<th>No. CIE positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>10</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alpha-hemolytic streptococci (not <em>S. pneumoniae</em>)</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No growth</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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**TABLE 3. Detection of purified pneumococcal capsular polysaccharide in phosphate-buffered saline by CIE and Phadebact**

<table>
<thead>
<tr>
<th>Pneumococcal serotype</th>
<th>Lowest CIE detection level (ng/ml)</th>
<th>Lowest Phadebact detection level (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>6A</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>6B</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>10,000*</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>14</td>
<td>10,000*</td>
<td>50</td>
</tr>
</tbody>
</table>

*Pneumococcal serogroups 7 and 14 were not detectable by CIE; therefore, immunodiffusion was used.*
of possible pneumococcal agents to provide laboratory information which is clinically relevant.

The possibility of direct agglutination of antigens in the CSF for the detection of pneumococcal meningitis is even more critical. Although in our study only 10 known pneumococcus-positive CSFs were tested, we found a 70% sensitivity rate compared with 40% for CIE and 100% specificity with 30 CSFs, 5 of which were negative for any growth and 4 of which grew alpha-hemolytic streptococci other than S. pneumoniae. Although cross-reactivity between certain pneumococcal and H. influenzae antigens has been reported (3, 5), none of the eight CSFs positive for pneumococcal and H. influenzae type b could be detected by the Phadebact coagglutination method. Purified pneumococcal polysaccharide in various amounts was analyzed by Phadebact, CIE, and immunodiffusion. The Phadebact coagglutination method was consistently able to detect smaller amounts of pneumococcal polysaccharide antigen in the range of 25 to 50 ng/ml, including serogroups 7 and 14.

The availability of a commercial test for the definitive identification of S. pneumoniae enables all laboratories to report final results within 1 day of receipt of specimen, providing more relevant use of the clinical laboratory. Direct coagglutination of pneumococcal antigen in CSF adds one more organism to the list of major pathogens causing bacterial meningitis which can be detected by this means. The addition of a coagglutination test for Neisseria meningitidis to those for S. pneumoniae, group B streptococci, and H. influenzae type b would complete the list, providing a rapid, simple, and more sensitive technique for the direct detection of the four major pathogens.

LITERATURE CITED