Latex Agglutination Test for Rapid Detection of Bacterial Antigens in Body Fluids

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ABSTRACT

A modified latex agglutination (LA) test performed on glass slides was evaluated for the detection of Streptococcus pneumoniae (Pn), Hemophilus influenzae type b (Hib), and Kj antigens in serum, cerebrospinal fluid (CSF), nasopharyngeal secretions, and other body fluids in patients suspected of having infection. Forty-eight, 61, and 31 specimens were tested for the presence of Pn, Hib, and Kj antigens, respectively, by countercurrent immunoelectrophoresis (CIE) and LA. Of the specimens positive by CIE or LA, 16 of 28 specimens (57 percent) were positive for Pn antigen by CIE while all 28 (100 percent) were positive by LA. LA was unique in detecting type 7 and 14 Pn antigens which are not detected by CIE. Twenty-seven of 37 specimens (73 percent) were positive for Hib by CIE, while all (100 percent) were positive by LA. LA test (11 of 11) was superior (p < 0.002) to CIE (4 of 11) in detecting Hib antigens on patients with nonmeningitic Hib diseases (cellulitis, epiglottitis and pneumonia). Sensitivity of CIE and LA were identical in detecting Kj (5 of 5) antigen. LA is a simple, sensitive and specific test for the detection of Pn, Hib, and Kj antigens in various body fluids.

Introduction

The need for improved diagnostic tools in infectious disease is well recognized. CIE is now extensively used for the detection of bacterial antigens in body fluids in a variety of infections.5,6,10,11,14,17,20 Recently, the LA test has been utilized for the detection of bacterial antigens in body fluids.7,9,15,18,19,22,23,24 This study was undertaken to evaluate the sensitivity and specificity of the LA test in the detection of bacterial antigens in various body fluids.

Materials and Methods

CIE and LA tests were performed on serum, cerebrospinal fluid, nasopharyn-
geal secretions, sputum, transtracheal aspirate, urine, joint, and pleural fluids from patients (age one day to 18 years) suspected of having infections. Nasopharyngeal secretions were liquified by diluting 1:1 with N-acetyl cysteine before CIE or LA tests were performed. Other specimens were used without any dilution or heat inactivation. Final bacteriologic diagnosis was based on: (1) bacterial isolation from cerebrospinal fluid, blood, pleural or joint fluid, or (2) the demonstrated presence of bacterial antigen in any of the body fluids by CIE in a patient with clinical symptomatology. A diagnosis of pneumonia was made if a child had either a positive blood culture or pneumococcal or Hib antigen detected in serum by CIE or fulfilled all of the following criteria: (1) physical and radiologic evidence of pneumonia; (2) fever > 38.5°C; (3) defervescence within 48 hours of initiation of therapy with a penicillin; and (4) an absolute neutrophil count of > 10,000 per mm³ and/or > 500 bands per mm³.

Countercurrent Immunoelectrophoresis (CIE)

CIE was carried out on 1 × 3" glass slides covered with 3 to 3.5 ml of one percent agarose in veronal buffer pH 6.6, 0.05M. Buffer used in the chamber was also 0.05 M veronal buffer, but the pH was 8.2. Parallel rows of wells, 3 mm in diameter were cut 5 mm apart in the agar. Each slide accommodated nine pairs of wells. Body fluid specimens to be tested filled the wells on the cathode side and the antisera were placed on the anode side. Electrophoresis was carried out at room temperature using 11 milliamps per slide. Slides were taken out of the chamber after three hours and read for precipitin line between the wells. Slides were again read the next day for precipitin line after overnight refrigeration in a moist chamber. This CIE system routinely was able to detect 12.5 nanogram per ml of polyribophosphate (PRP).

Latex Particle Agglutination (LA) Test

Latex particles (0.8 μ in diameter) were sensitized with pneumococcal antisera* diluted 1/10 or K₁ antisera† diluted 1/10, or *H. influenzae* type b (Hib) antisera† diluted 1/16. Glycine buffered saline (0.1M glycine in one percent sodium chloride, pH 8.2) was used as diluent for the antisera. Equal parts of suspended latex particles and diluted antisera were incubated at 37° for three hours and then kept at 4° overnight. Finally, two parts of 0.1 M glycine in one percent sodium chloride with 0.1 percent bovine serum albumin was added; the preparation was refrigerated until used. Final dilutions of antisera latex suspensions were 1:40 for pneumococcus and K₁ antisera, and 1:64 for Hib antisera. Control latex suspensions were prepared in a similar manner using either normal horse serum (for K₁ antigen control) or rabbit serum (for pneumococcal and *H. influenzae* type b antigen control).

The LA test was performed in two ways. The standard test was carried out utilizing an agglutination plate. Two drops of the specimen together with one drop of either sensitized latex or the control were deposited in each ring with a Pasteur pipet. The plate was then agitated gently for three minutes and read macroscopically. In the modified LA test, a regular 1 × 3" glass slide was used in place of the agglutination plate. Materials were added to the slide as described previously, gently mixed, and, finally, a coverslip was added. These preparations were also read after three minutes, first macroscopically and then under low power magnification (50 to 100 ×) if necessary for the agglutina-

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*Omnisera obtained from Statens Serum Institut, Denmark.
† Kindly supplied by John Robbins, M.D.
‡ Obtained from Hyland Laboratories.
tion of the latex particles. Routine detection showed 1.5 nanogram per ml of PRP.

Results

As shown in table I, CIE and LA tests were performed on 48 specimens for pneumococcal antigen, 61 specimens for H. influenzae type b (Hib) antigen, and 31 specimens for Kt antigen.

Of a total of 48 specimens tested for pneumococcal antigens, 16 were positive by both CIE and LA; none were positive by CIE and negative by LA. Twelve specimens were negative by CIE and positive by LA. From these 12, 10 came from patients with pneumonia, one from a patient with meningitis (culture proven) and one with arthritis (culture proven). In 50 percent (8/16) of the patients who fulfilled the diagnostic criteria for Streptococcus pneumoniae pneumonia, antigen was detected by both CIE and LA. In the remaining eight, antigen could not be detected by CIE; however, LA test was positive in the sera in three patients, in the nasopharyngeal in three patients, and in both serum and nasopharyngeal secretion two patients, respectively. Patients in this pneumonia group who had a negative CIE but positive LA test had Streptococcus pneumoniae recovered in their blood culture.

Types 7 and 14 Streptococcus pneumoniae were isolated in one and four blood cultures, respectively, where LA was positive but no antigen was detected by CIE. Types 7 and 14 pneumococcus are not routinely detected by CIE because their antigens do not carry the proper electrical charge, and they migrate in the wrong direction. accepting these limitations of CIE, seven specimens (one serum from meningitis, one nasopharyngeal from arthritis, two serum and three nasopharyngeal from pneumonia patients) were positive for pneumococcal antigen by LA and not by CIE. Twenty specimens from patients with non-

pneumococcal disease were negative by both methods. These specimens belonged to patients with aseptic meningitis (three cerebrospinal fluid), culture proven Hemophilus influenzae type b disease (six sera, two cerebrospinal fluid, two nasopharyngeal, one pleural fluid and one urine), E. coli meningitis (one serum and one cerebrospinal fluid) and patients with bronchiolitis (three nasopharyngeal). Results of agglutination-plate and glass-slide latex tests were identical.

Twenty-seven out of a total of 61 specimens tested for H. influenzae type b (Hib) antigen were positive by both CIE and LA. Three specimens were positive by CIE and negative by LA. These were falsely positive as they were obtained from patients proven by culture to have Streptococcus pneumoniae meningitis. Ten specimens were negative by CIE and positive by LA. Twenty-one specimens from patients with non H. influenzae type
TABLE II

<table>
<thead>
<tr>
<th>Disease</th>
<th>CIE No. Positive</th>
<th>Negative</th>
<th>LA No. Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningitic</td>
<td>26</td>
<td>23</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>Non-meningitic</td>
<td>11</td>
<td>4*</td>
<td>7</td>
<td>11*</td>
</tr>
</tbody>
</table>

*p < 0.002

b diseases were negative by both methods. These specimens belonged to patients with aseptic meningitis (eight cerebrospinal fluid), culture proven pneumococcal disease (two cerebrospinal fluid, five sera and three nasopharyngeal) and E. coli meningitis (three cerebrospinal fluid).

Of a total of 31 specimens tested for K1 antigen, five were positive by both CIE and LA. Three specimens were positive by CIE and negative by LA. These were false positives as they were obtained from patients proven by culture to have H. influenzae type b meningitis. None of the specimens were negative by CIE and positive by LA. Twenty-three specimens were negative by both methods. These specimens belonged to patients with aseptic meningitis (eight cerebrospinal), culture proven pneumococcal disease (two cerebrospinal fluid, three sera) culture proven Hib disease (three cerebrospinal fluid, two sera) and healthy premature infants (five cerebrospinal fluid) born after premature rupture of membranes. The results of the LA test done on agglutination-plates and glass slides were identical.

CIE vs LA in Meningitis and Nonmeningitic Hib Disease

When the data from patients with Hib disease were divided into patients with meningitic or nonmeningitic disease, it became evident that LA was more sensitive than CIE for detection of Hib antigen in body fluid specimens from patients with nonmeningitic Hib disease (table II). Twenty-three of 26 specimens from 15 patients with culture proven Hib meningitis were positive by CIE while all 26 were positive by LA. Four of 11 specimens from eight patients with nonmeningitic H. influenzae type b disease (four pneumonia, two epiglottitis, two cellulitis) were positive by CIE, while all 11 specimens were positive by LA for this antigen. This difference is statistically significant (p < 0.002). These seven (CIE−, LA+) specimens belonged to six patients with culture proven H. influenzae disease (one serum and one nasopharyngeal secretion from two patients with acute epiglottitis, one serum and one nasopharyngeal secretion from two patients with facial cellulitis, and two sera and one pleural fluid from two patients with pneumonia).

Discussion

Whittle et al found the LA test to be as sensitive as CIE and more sensitive than Gram stain or culture in the diagnosis of H. influenzae, Neisseria meningitidis and Streptococcal pneumoniae meningitis.24 Scheifele et al and Ward et al found the latex test to be more sensitive and specific in Hib infection in primate models and human disease respectively, while Coonrod and Bauer found LA to be less sensitive than CIE for the demonstration of pneumococcal antigen.

In the present study, the LA test was found to be more sensitive than CIE, especially for detection of pneumococcal and H. influenzae type b antigens. Approximately 66 percent (4/6) of specimens of nasopharyngeal secretions and 50 percent (8/16) of specimens of serum from patients with pneumococcal disease were positive only by the LA test. The LA test was unique in detecting types 7 and 14.
pneumococcal antigens, which are not detected by conventional CIE because of their electrical charge. The LA test was also more sensitive than CIE in detection of Hib antigen. Approximately 30 percent (4/14) of serum specimens and 50 percent (3/6) of nasopharyngeal secretions were positive only by the latex test for Hib antigen. This was especially so in nonmeningitic Hib disease where approximately 63 percent (7/11) of the specimens were positive only by the LA test.

Congeni and Nankervis have demonstrated the value of detecting pneumococcal and Hib antigens by CIE in nasopharyngeal secretions in the diagnosis of pneumonia.8 The majority of pneumonia patients who were positive by LA but negative by CIE were cases of type 14 Streptococcus pneumoniae. Type 14 pneumococcus has repeatedly been shown to be one of the common type causing pneumonia in children.1,12,18 The fact that this type cannot be detected by routine CIE represents a major drawback of CIE that can be overcome by LA.

In this study, the results of the LA tests done on agglutination plates and glass slides were identical. Low power magnification was used for reading the glass slides when the latex test appeared weakly positive by unaided eye. Low power magnification was helpful in distinguishing the positive tests with the negative controls. The glass slide latex agglutination test is easy to perform, and reliable results can be obtained as long as proper controls are run simultaneously.

Using commercially obtained antisera with the latex test, the present authors were routinely able to detect 1.5 nanogram per ml of polyribitol-phosphate (PRP) while CIE was sensitive for 12.5 nanogram per ml of PRP. It has been suggested that CIE measures only the PRP antigen whereas the latex agglutination test detects other antigens in addition.24 Three specimens from patients with culture proven Streptococcus pneumoniae meningitis and three specimens from patients with culture proven cases of Hemophilus influenzae type b gave false positive results with CIE for PRP and K1 antigens, respectively. Several Gram positive and negative bacterial pathogens contain PRP antigen.5,4 Type 15 Streptococcus pneumoniae was isolated from one of the cerebrospinal fluid specimens, giving a false positive reaction for PRP. Pneumococcal isolates from the other two patients ("CIE False+, LA-") were not typed. Capsular polysaccharides of types 15, 29, and 34 are known to cross react with Hib antigens.3,16,25

In 1971, Bradshaw et al4 demonstrated a partial identity reaction between Hib PRP and capsular polysaccharide of E. coli. Even though these false positives observed with CIE can be explained on the basis of antigens cross reactivity, no false positive reactions occurred in latex agglutination tests. Some investigators have reported false positive reactions with the LA tests on various body fluids including CSF.23 This problem was not encountered by us. Several of our negative cerebrospinal fluids had high protein content either because of being obtained from patients with aseptic meningitis or from premature newborns. This elevated protein content did not result in nonspecific agglutination or precipitin lines on LA and CIE tests, respectively. Sensitivity of CIE and LA tests vary from institution to institution depending on such factors as the type of antisera and the pH and ionic strength of the buffers used. The sensitivity of CIE can be increased by utilizing a more sensitive gel support system, concentrating the specimens, or reading the slides after several hours of cooling. These procedures are time consuming and tedious. The LA test provides the answer within minutes and is easy to perform. When possible, both the methods should be used since that may reduce the number of false positives by any one of the methods.
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References


