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Use of the Directigen Latex Agglutination Test for Detection of *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis* Antigens in Cerebrospinal Fluid from Meningitis Patients

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Cerebrospinal fluid specimens from 257 persons were tested for the presence of bacterial antigens by counterimmunoelectrophoresis and the Directigen meningitis test (Hynson, Westcott & Dunning, Div. Becton Dickinson & Co., Baltimore, Md.). The specimens were obtained from 162 patients with meningitis caused by *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, or *Neisseria meningitidis* serogroups A and C and from 95 patients without bacterial meningitis or meningitis caused by other bacterial agents. Directigen detected *H. influenzae* type b antigen in 83% (69 of 83) of the specimens obtained from patients with *H. influenzae* disease, pneumococcal antigen in 77% (30 of 39) of the specimens from patients with pneumococcal disease, and *N. meningitidis* antigen in 93% (37 of 40) of the specimens from patients with disease caused by *N. meningitidis* serogroups A and C. The comparable figures for counterimmunoelectrophoresis were 66% (55 of 83), 79% (31 of 39), and 78% (31 of 40), respectively. No false-positive reactions were reported with the Directigen reagents. Nonspecific reactions (agglutination with more than one of the four Directigen latex reagents) were noted with five specimens. The nonspecific reactions were resolved in four of the five specimens by heating (100°C for 3 min). The accumulated data demonstrate that the sensitivity of the Directigen meningitis test is better than or at least equivalent to the sensitivity of counterimmunoelectrophoresis for the detection of antigens in cerebrospinal fluid.

There is currently considerable interest in applying immunodiagnostic procedures to the rapid detection of microbial antigens in clinical specimens. This approach to the diagnosis of infectious diseases has been spurred by the development of techniques that are simple and, depending on the quality of the reagents used (5, 6), can be both sensitive and specific. Immunological detection of bacterial antigens is particularly applicable to cerebrospinal fluid (CSF) obtained from bacterial meningitis patients, since high concentrations of antigen are generally found in these specimens. The acute nature of meningococcal disease and the desire for better patient management have also increased the interest in the extremely rapid diagnoses, which are feasible with immunological techniques. The method that has been most thoroughly evaluated for this purpose is counterimmunoelectrophoresis (CIE) (1, 2, 4, 7, 8, 10). Latex agglutination, however, is generally considered to be more sensitive than CIE (V. Corasaniti, XIII Int. Congr. Microbiol., Boston, Mass., 8 to 13 August 1982; 3, 8–11). The study reported here compares the Directigen latex agglutination test to CIE for the detection of *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, and *Neisseria meningitidis* groups A and C bacterial antigens in CSF specimens. The results demonstrate that the Directigen meningitis kit is frequently more effective than CIE for the detection of bacterial antigens present in CSF specimens from patients with meningitis.

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MATERIALS AND METHODS

Specimens. CSF specimens were obtained from 162 patients with *H. influenzae*, pneumococcal, or meningococcal meningitis at the time of admission to the medical institutions represented by the authors. The identity of the bacterial agents present in the specimens was determined by culture or detection of specific antigen by CIE or coagglutination (9). Eighty-three CSF samples were from patients with *H. influenzae* type b meningitis, 39 were from patients with pneumococcal meningitis, 37 were from patients with group A meningococcal meningitis, and 3 were from patients with group C meningococcal disease. The numbers of culture-positive specimens in the four etiological groups were 66, 30, 22, and 0, respectively. There were also 95 negative control specimens that were antigen negative or that contained agents other than those detected with the Directigen and CIE reagents. Specimens that were not tested for the presence of antigen either by CIE or Directigen immediately after collection were stored at −20 or −70°C.

Immunoassays. The specimens were tested for the presence of bacterial antigens by the following procedures.

(i) CIE. CIE was performed in agarose made up in barbital

buffer (pH 8.6) with immune rabbit sera obtained from Hyland Diagnostics (Deerfield, Ill.) and Difco Laboratories (Detroit, Mich.) for *H. influenzae* type b, from Statens Seruminstitut (Copenhagen, Denmark) for *S. pneumoniae*, and from N. Vedros, Naval Bioscience Laboratory (Oakland, Calif.) and Wellcome Diagnostics (London, England) for *N. meningitidis* groups A and C, respectively. CIE procedures varied at each medical institution, but all procedures complied with routinely accepted techniques (1).

(ii) Directigen latex agglutination. Directigen latex agglutination was performed with the Directigen meningitis test by the procedure recommended by the manufacturer (Hynson, Westcott & Dunning, Div. Becton Dickinson & Co., Baltimore, Md.). A 50-μl sample of specimen was individually mixed with 15 μl of each of the four Directigen latex reagents in circles measuring 18 mm in diameter on a glass slide. The slides were rocked a few times and then placed on a mechanical rotator at an approximate speed of 100 rpm for 10 min. Any agglutination within the circles as seen against a dark background was recorded as positive.

Positive and negative controls supplied by the manufacturer were included in each batch of specimens.

(iii) Other assays. The Bactigen latex agglutination test (Wampole Laboratories, Cranbury, N.J.) for *H. influenzae* type b was used by mixing 10 μl of reagent with 50 μl of CSF on a glass slide and rotating for 10 min according to the manufacturer's directions. The Phadebact *Haemophilus* and *Pneumococcus* coagglutination tests (Pharmacia Diagnostics, Piscataway, N.J.) were employed by mixing a drop of CSF and a drop of reagent on a slide and rocking the slide for 30 s. Before the performance of the Phadebact test, all specimens were heated to 80°C for 10 min. The Bactigen and Phadebact kits were also tested with the controls recommended by the manufacturers.

Statistical analysis. The paired *t* test (corrected for continuity when appropriate) was used to determine whether there was a statistically significant difference between the number of specimens in which antigen was detected by the different immunoassay systems.

RESULTS

Directigen detected specific antigen in 69 (83%) of 83 *H. influenzae* specimens, 30 (77%) of 39 pneumococcal specimens, and 37 (93%) of 40 meningococcal specimens. The comparable figures for CIE were 55 (66%), 31 (79%), and 31 (78%), respectively (Table 1). Although there was no significant difference in the two methods for detecting pneumococcal antigens, Directigen was more efficient than CIE in detecting *H. influenzae* type b ($P < 0.001$) and *N. meningitidis* group A ($P < 0.01$) antigens in CSF specimens. If only the culture positive specimens are considered, Directigen detected antigen in 86% of the *H. influenzae* specimens, 80% of the pneumococcal specimens, and 91% of the meningococcal CSFs, whereas the comparable figures for CIE were 70, 77, and 73% respectively (Table 2). Again, the superior performance obtained with Directigen with the culture-positive *H. influenzae* ($P < 0.01$) and group A meningococcal ($P = 0.05$) specimens was significant.

Of the 132 *H. influenzae*, pneumococcal, and meningococcal specimens that were cultured at the time of collection, 118 were positive; 101 of the culture-positive CSFs were positive by Directigen, and 85 were positive by CIE. Nine of the culture negative specimens (two *H. influenzae* and seven group A meningococcal specimens) were positive by Directigen, and seven (one *H. influenzae*, one pneumococcal, and five group A meningococcal specimens) were positive by CIE.

TABLE 1. Comparison of Directigen and CIE for detection of specific antigens in CSF[a]

Specimen	No. tested	Directigen positive	CIE positive
H. influenzae type b	83	69	55
S. pneumoniae	39	30	31
N. meningitidis group A	37	35	29
N. meningitidis group C	3	2	2

[a] Each specimen was culture positive or positive by immunoassay (CIE, coagglutination) or both before this study.

One negative specimen, two specimens positive by culture for *H. influenzae*, and two specimens containing meningococcal antigen produced agglutination with two or more Directigen reagents. These "uninterpretable reactions" were eliminated in four of the five specimens by heating the CSF at 100°C for 3 min before retesting. After heating, the appropriate latex reagent detected the antigen in each specimen. The volume of the fifth specimen was not sufficient to allow retesting after heating.

None of the 95 specimens that were obtained from patients without bacterial meningitis or from patients with meningitis caused by bacterial agents other than those detected by Directigen reacted with any of the Directigen reagents; 65 of these specimens were negative by culture for bacterial agents, and 30 were positive for the agents listed in Table 3. The specimens were also negative by CIE for the antigens of *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis* groups A and C.

A number of the specimens positive for *H. influenzae*, pneumococcal or meningococcal antigens were also tested with the appropriate Bactigen and Phadebact reagents. Of 30 *H. influenzae* type b-containing CSF specimens tested with both the appropriate Directigen and Bactigen reagents, 29 were both Directigen and Bactigen positive, whereas 1 was Bactigen positive and Directigen negative. Phadebact detected antigen in 29 of the 44 *H. influenzae* type b specimens that were tested with both Phadebact and Directigen reagents. A significantly greater number of specimens (35 versus 29) were positive by Directigen than by Phadebact ($P < 0.05$). Of 21 pneumococcal specimens tested with both Directigen and Phadebact reagents, 16 were positive with Phadebact, whereas 18 were positive with Directigen.

DISCUSSION

A number of studies have been conducted to determine the relative sensitivity of latex agglutination and CIE in detecting the various microorganisms capable of inducing bacterial meningitis. Through a comparison of the data obtained in these earlier studies (in particular, Denis et al. [3]), it is clear that the latex agglutination method described in this and earlier work is at least as effective as CIE in

TABLE 2. Comparison of Directigen and CIE for detection of specific antigens in culture-positive CSF specimens

Specimen	No. tested	Directigen positive	CIE positive
H. influenzae type b	66	57	46
S. pneumoniae	30	24	23
N. meningitidis group A	22	20	16

TABLE 3. Culture-positive specimens tested by CIE and Directigen

Organism	No. tested
Escherichia coli	3
Group B streptococcus	4
Group D enterococcus	1
Klebsiella oxytoca	2
Klebsiella pneumoniae	1
Litteria monocytogenes	1
Neisseria meningitidis group B	4
N. meningitidis group Y	2
N. meningitidis (not groups A or C)	4
Staphylococcus aureus	1
Staphylococcus epidermidis	2
S. epidermidis and group D. enterococcus	2
S. epidermidis and unidentified yeast	1
Sarcina lutea	1
Viridans streptococci	1

detecting antigen from *H. influenzae* type b, *S. pneumoniae*, and the A and C groups of *N. meningitidis*. In this study, the Directigen latex reagents were more effective than CIE in detecting *H. influenzae* type b and *N. meningitidis* group A antigen, whereas the latex reagents were as effective as CIE in detecting pneumococcal antigens in the appropriate specimens.

The reactions obtained with the latex reagents contained in the Directigen meningitis kit were generally rapid and pronounced. No false-positive reactions were produced when the reagents were used to test CSFs that either contained no identifiable microorganisms or that contained microorganisms other than those recognized by the reagents in the kit. Uninterpretable agglutination (defined as the agglutination of two or more Directigen latex reagents with the same clinical specimen) was produced by 2% of the CSFs tested. These uninterpretable reactions can easily be eliminated by heating the specimens. Uninterpretable reactions with latex reagents may be a more significant problem with specimens other than CSF.

Of the 162 CSFs from *Haemophilus*, pneumococcal, and meningococcal meningitis patients, 83 were tested by CIE both at the time of specimen collection and a second time for this study (i.e., after prolonged storage); 21 of the 75 specimens that were positive when collected were negative after storage (data not shown). This suggests that there was antigen deterioration in some of the specimens and that both the Directigen and CIE results would have been superior to those reported here if fresh specimens were tested.

The efficacy of the Wampole Bactigen *H. influenzae* type b reagent for detection of specific antigen in CSF was essentially identical to that of the corresponding reagent in the Directigen meningitis kit. Similarly, the Directigen and Phadebact pneumococcal reagents were equally efficacious at detecting specific antigen in the clinical specimens. However, the Phadebact coagglutination test reagent was significantly less effective than the corresponding Directigen reagent for the detection of *H. influenzae* antigen.

The Directigen meningitis test is an effective method for rapidly diagnosing meningitis caused by *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis* groups A and C. Since only reagents and glass slides are used, the test can be performed in any laboratory, and since the test does not require viable microorganisms to assure an appropriate reaction, it can detect antigen even in specimens that were stored or transported before testing. These and the other advantages of using an antigen detection system make the Directigen meningitis test an excellent adjunct to culture in the diagnosis of bacterial meningitis.

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