Comparative Evaluation of Three Commercial Products and Counterimmunoelectrophoresis for the Detection of Antigens in Cerebrospinal Fluid

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Three commercial products and counterimmunoelectrophoresis were evaluated for their ability to detect microbial antigens of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in cerebrospinal fluid from 157 patients suspected of having meningitis. Thirty-four patients were diagnosed as having bacterial meningitis by culture, microscopy, or antigen detection. The overall results showed the following detection percentages: counterimmunoelectrophoresis, 76%; Phadebact CSF, 76%; Directigen, 82%, and Bactigen, 93%. The results with purified antigen revealed that latex agglutination was more sensitive than coagglutination, which in turn was more sensitive than counterimmunoelectrophoresis.

The rapid detection of soluble bacterial antigens in the cerebrospinal fluid (CSF) of patients with meningitis has distinct clinical advantages. A positive result can allow more precise antimicrobial therapy. The results of antigen detection tests are available at least 18 h before culture results. These tests may give positive results even when Gram stain examination and culture are negative. Furthermore, the amount of antigen in the CSF may provide valuable prognostic information (8).

Several methods are available for antigen detection. They include counterimmunoelectrophoresis (CIE), latex agglutination (LA), coagglutination (COA), enzyme-linked immunosorbent assay, and radioimmunoassay. The last two methods are time-consuming (2 to 4 h) and are most cost efficient when specimens are processed in batches. CIE is technically difficult, requires special equipment, and is reported to be less sensitive than LA and COA for some microbial antigens (8, 10).

The most rapid antigen tests available are LA and COA. Both suffer from possible false-positive results due to non-specific agglutination of indicator particles by untreated biological specimens or biological cross-reactions. Until recently, comprehensive panels of reagents which detect the most common agents of bacterial meningitis were not commercially available.

Drow et al. (4), however, have recently reported that the Phadebact CSF product, containing individual antisera to the four most common agents of bacterial meningitis, detected 74% of the positive specimens, compared with 65% detected by CIE. Ingram et al. (6) tested the Wellcogen (Wellcome Diagnostics) LA test for the detection of *Haemophilus influenzae* b, *Streptococcus pneumoniae*, and *Neisseria meningitidis* groups A, C, Y, and W135 in CSF, serum, and urine. They reported that the Wellcogen LA tests were more sensitive than both CIE and some individual LA and COA kits (e.g., Bactigen for *H. influenzae* b, Phadebact for *S. pneumoniae*).

This study evaluated the accuracy of the Directigen (Hynson, Westcott, & Dunning), Phadebact CSF (Pharmacia, Inc.), Bactigen (Wampole Laboratories), and CIE tests with CSF specimens collected from patients with clinical signs and symptoms of bacterial meningitis.

MATERIALS AND METHODS

CSF specimens for analysis were chosen from those sent to the clinical laboratory for routine microbiological processing. They represented both specimens referred to and inpatient specimens from the John Dempsey Hospital, University of Connecticut Health Center. After routine processing in which each CSF sample was examined microscopically, cultured, and tested for bacterial antigens by CIE, the specimens were frozen at −70°C for later analysis. All CSF specimens positive for *N. meningitidis* group B were tested immediately and not frozen.

To be included in the study as a "positive" specimen, two of three tests (Gram stain, culture, or antigen detection) had to yield a positive result.

Culturing procedures. CSF samples were routinely cultured on 7.5% blood agar and on chocolate agar. The plates were incubated in a CO_2 incubator for a maximum of 48 h. Colonies were identified as described previously (9).

CIE. CIE was done as described previously (11), with 1% agarose-coated Mylar film, a Bioware electrophoresis chamber, and antiserum. *H. influenzae* b antiserum was from Hyland, Inc., and *S. pneumoniae* antiserum (Omniserum) was from the Statenserum-institut, Copenhagen, Denmark. The purified polysaccharides of *H. influenzae* b (obtained from D. H. Smith, University of Rochester), *N. meningitidis* groups A and C (meningococcal vaccine; Merck Sharpe & Dohme), and *S. pneumoniae* (Pneumovax) were used both as control antigens and to determine the sensitivity of the kits. All CIE films were stained wth Coomassie blue as described previously (11).

LA and COA. The LA and COA tests were done on CSF samples as recommended by the manufacturers. Agglutination was rated from 1+ to 4+ as follows: 1+, very slight, fine granular agglutination; 2+, moderate agglutination with uniform small clumps; 3+, heavy agglutination with uniform large clumps; 4+, heavy agglutination with large clumps widely separated.

Heat treatment of CSF specimens. When the sensitized latex particles agglutinated with more than one antiserum, the CSF specimen was designated "uninterpretable" and heated at 100°C for 3 min (for Directigen) to eliminate nonspecific agglutination before retesting. CSF specimens to be tested by COA (Phadebact CSF) were routinely incubated

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at 80°C for 5 min before testing. The Bactigen kit did not specify pre- or posttest heat inactivation of CSF samples to remove nonspecific agglutination. The antisera available in each kit are listed in Table 1.

Sensitivity of test methods. The purified antigens described above for the CIE procedure were suspended in membrane-filtered CSF, diluted serially 10-fold, and tested by CIE, LA, and COA. An endpoint was defined as 3+ to 4+ agglutination by LA or COA and as a visible precipitin line before staining by CIE.

Controls. All CIE assays were controlled by including known polysaccharide antigens on each Mylar sheet. Both positive and negative controls on LA and COA were used as suggested in the package inserts of the individual products.

RESULTS

The results of the commercial antigen detection systems and CIE with culture of the organism from CSF are shown in Table 2. Not all samples were tested with every kit because of the limited amounts of CSF available. Of the nine patients with *N. meningitidis* meningitis, five harbored group C and four harbored group B meningococci. Although all the group B meningococci cases were culture positive, none of these antigens was detected by CIE and 80% were detected by the Directigen monoclonal antibody reagent. The CIE test was positive for 100% and Directigen was positive for 75% of the culture-positive *N. meningitidis* group C cases. The Bactigen and Phadebact CSF kits contained polyvalent meningococcal antisera and detected 66 and 50% of the patients with culture-positive meningococcal disease, respectively. The four isolates missed by the Phadebact CSF test and the two isolates missed by the Bactigen test were all *N. meningitidis* group B.

Seven patients had pneumococcal meningitis (Table 2). Six were culture positive (86%), five (71%) were detected by CIE, six (86%) were detected by Phadebact CSF, and seven (100%) were detected by Directigen and Bactigen. The one patient from whom no *S. pneumoniae* could be cultured showed gram-positive lancet-shaped diplococci in the Gram stain of the CSF, and the CSF ultimately revealed pneumococcal antigen.

The results for 18 patients with *H. influenzae* meningitis, 17 of whom were confirmed by culture, are shown in Table 2. Again, the one patient from whom no organisms were isolated had a characteristic CSF Gram stain, and *H. influenzae* antigen was detected. It is also important to note that the CSF samples from three of the four (75%) patients who were not tested by the Bactigen reagents because of insufficient amounts of CSF gave positive results when tested by Directigen, Phadebact CSF, and CIE; they were also culture positive. One patient's CSF was positive by culture, CIE, and Phadebact CSF, but negative by Directigen. Unfortunately, there is no way to predict what the Bactigen results would have been if sufficient sample had been available. The

percentage of all specimens positive by a specified method is also presented in Table 2.

All the commercial kits suggest recording semiquantitative agglutination reactions from 1+ to 4+. The mean semiquantitation values obtained for all specimens by Directigen, Bactigen, and Phadebact CSF were 3.2+ for Directigen and Bactigen (LA methods) and 2.7+ for Phadebact CSF (COA method). There was a statistically significant difference (Student's *t* test) between the two values ($P < 0.05$).

All the methods evaluated proved to be relatively specific; 123 CSF specimens (Table 2) which yielded no organism on culture were negative by all of the methods. There were exceptions, however: one CSF specimen which contained *N. meningitidis* group Y gave a precipitin line by CIE with both *N. meningitidis* groups A and B as well as with group Y antiserum. Another patient whose CSF culture was CIE-positive for *H. influenzae* b was negative by LA, COA, and culture. Two specimens tested by Directigen yielded false-positive results. In one of these specimens, *H. influenzae* was isolated and agglutination was observed not only with *H. influenzae* b antiserum (2+), but also with *S. pneumoniae* antiserum (1+). The other specimen which contained *N. meningitidis* group B yielded a 1+ agglutination reaction with antiserum to *S. pneumoniae* when tested by Directigen. Heating at 100°C for 3 min did not remove this activity. No false-positive reactions were observed with the Phadebact CSF kit. No nonspecific agglutination was observed with Directigen, Bactigen, or, after preincubation at 80°C for 5 min, with Phadebact CSF.

The sensitivity of the three antigen detection systems with purified capsular polysaccharide from *S. pneumoniae*, *N. meningitidis* (groups A and C), and *H. influenzae* is shown in Table 3. With few exceptions, Directigen (LA) was the most sensitive, followed in order by Bactigen (LA), Phadebact CSF (COA), and CIE.

DISCUSSION

The availability of comprehensive LA and COA kits for detecting bacterial antigen in CSF is a major advance in the diagnosis of meningitis. Until now there have been individual reagents but no single kit to detect the microorganisms which most commonly cause bacterial meningitis. Similarly, although many laboratories have used CIE for years to detect antigens in CSF, there has never been a concerted effort to market a CIE product for that specific purpose. Although there are differences among the methods tested, the use of any of them gives a significant advantage in diagnosing meningitis. Of prime importance is the rapidity of diagnosis compared with culture methods. Although the CSF Gram stain is also rapid and sensitive, it relies heavily on the skill of the microscopist for maximum specificity. A sensitive, specific diagnosis of bacterial meningitis caused by *N. meningitidis*, *H. influenzae*, or *S. pneumoniae* offers significant clinical advantages both in focusing antimicrobial

TABLE 1. Sensitized antisera available in commercial antigen detection kits

Product	S. pneumoniae[a]	H. influenzae						N. meningitidis				
		A	B	C	D	E	F	A	B	C	Y	W135
Directigen	X		X					X	X[b]	X		
Phadebact CSF	X	X	X	X	X	X	X	X	X	X	X	X
Bactigen	X		X					X	X	X	X	

[a] Polyvalent, 83 types.
[b] Separate monoclonal antibody.

TABLE 2. Comparison of CIE, culture, and commercial agglutination products for antigen detection in CSF

Organism	No. of patients	No. of positive specimens/no. tested (%)[a]				
		Culture	CIE	Directigen	Bactigen	Phadebact CSF
N. meningitidis						
Group B	5	5/5 (100)	0/5 (0)	4/5 (80)	4/6 (66)[b]	4/8 (50)[b]
Group C	4	4/4 (100)	4/4 (100)	3/4 (75)		
S. pneumoniae	7	6/7 (86)	5/7 (71)	7/7 (100)	7/7 (100)	6/7 (86)
H. influenzae b	18	17/18 (94)	17/18 (94)	14/18 (78)	14/14 (100)	15/18 (83)
Totals	34	32/34 (94)	26/34 (76)	28/34 (82)	25/27 (93)	25/33 (76)

[a] No false-positive results were obtained by any of the methods for 123 culture-negative samples.
[b] Polyvalent antiserum used.

therapy and even in projecting the eventual outcome of the disease.

Although 94% of the cases of bacterial meningitis described in this report were confirmed by culture, the results of other studies (3, 4) show that antigen detection has a slight advantage over culture or Gram stain as a diagnostic tool, particularly for patients who have been pretreated with antibiotics. In those few cases when the Gram stain and culture of the CSF are negative, the detection of antigen in the CSF may be the sole diagnostic aid.

Although these products are currently not approved for other body fluids, many workers have used CIE, LA, and COA to detect antigen in serum and urine. Several reports confirm the diagnostic advantage of detecting antigen in urine as an adjunct to detecting it in CSF (2, 5, 7).

The advantages of LA and COA over CIE have been noted previously. The same advantages were seen in this study. The LA and COA kits are easier to use than CIE. Technologists need less training for their use, and there are fewer variables in the test procedure. The time required to perform agglutination tests (5 to 15 min) is significantly shorter than that for CIE (45 to 60 min). The staining of CIE films, which we consider necessary, requires an additional 18 h. Carey (1) has summarized the cost of CIE, commercial LA, and COA kits; the range is from $14.70 for the five antisera used in CIE to a per-test cost of $9.10 for Bactigen, $8.35 for Directigen, and $9.00 for Phadebact CSF. However, actual cost comparison is difficult because so many factors are involved, including individual hospital contract prices for kits, the number of specimens that can be processed simultaneously, and the shelf life of the reagents.

Most investigators have indicated that LA is more sensitive than either CIE or COA and that COA is more sensitive than CIE. This report confirms these results when the purified antigens and the reagents provided in the kits were used. However, CIE has been used in this laboratory for 15 years, and as performed clinically it is a very sensitive test. This is shown by the data (Table 2), i.e., 76% of all specimens were positive by CIE, compared with 76% by COA (Phadebact CSF) and 82% (Directigen) and 93% (Bactigen) of specimens, respectively, by LA. The sensitivity of detection of *H. influenzae* antigen was 100% with Bactigen, followed by 94% with CIE, 83% with Phadebact CSF, and 78% with Directigen. However, it must be reiterated that one of the four specimens analyzed by CIE, Directigen, and Phadebact CSF, but not by Bactigen, was positive with CIE and Phadebact CSF but negative with Directigen. Had the specimen also been negative with Bactigen, then the detection sensitivity of the Bactigen reagent would have decreased to 93%. Although it is unlikely that all four specimens not tested by Bactigen would have been negative if tested, the sensitivity of detection would have decreased to 78% if such a result had occurred. This clinical difference

between the two LA kits for *H. influenzae* is difficult to explain even though there is a 10-fold difference in the sensitivity of *H. influenzae* antigen detection between the Directigen and Bactigen tests.

Both LA kits detected 100% of the *S. pneumoniae* meningitis cases, whereas Phadebact (86%) and CIE (71%) were less effective. However, seven cases of pneumococcal meningitis may not be statistically significant. One case not detected by CIE had a type 14 *S. pneumoniae* infection.

Detection of *N. meningitidis* group B was the most difficult. Our laboratory uses a CIE system for *N. meningitidis* group B that includes Noble agar and *Escherichia coli* K1 antiserum, *E. coli* K1 being antigenically identical to *N. meningitidis*. Even so, CIE detected none of the five group B meningococcus cases. COA detected four of nine (44%). The Directigen reagents for group B meningococci are marketed in a separate kit that uses a monoclonal antibody. Of the five fresh specimens tested, four were positive (80%). Both Bactigen and Phadebact CSF use a polyclonal *N. meningitidis* antiserum, which may explain the reduced sensitivity observed.

Although the results with pure microbial capsular polysaccharide showed a difference in sensitivity between the methods, we also noted that semiquantitation of the antigen under clinical conditions by LA and COA showed that greater agglutination occurred with LA than with COA. These results were averaged for the three methods, and there was a statistically significant difference between the mean values for LA and COA. It is not clear whether this indicates that LA is more sensitive or that latex polystyrene particles are read more easily than staphylococcal particles.

Agglutination tests have some disadvantages, one of which is nonspecific agglutination. Although nonspecific reactions may occur with CIE, they are easily detectable as a haze around the wells or as a precipitin line that disappears after staining. Nonspecific agglutination is not a significant problem in CSF, but it is a problem in serum and urine. Of the 123 negative CSF specimens, there were no instances of nonspecific agglutination. When it does appear, it occurs more frequently in COA than in LA. For these reasons, the Phadebact CSF insert recommends incubating all specimens at 80°C for 3 min before testing. The Directigen product

TABLE 3. Sensitivity of antigen detection systems

Antigen	Antigen concn (ng/ml of sterilized CSF) detectable by:			
	Directigen (LA)	Bactigen (LA)	Phadebact CSF (COA)	CIE
S. pneumoniae	0.75	0.2	6.0	24.0
N. meningitidis group A	1.5	7.5	1.5	24.0
N. meningitidis group C	0.75	7.5	1.5	24.0
H. influenzae b	1.5	0.1	1.5	6.0

insert suggests heat treatment (100°C) only if spontaneous agglutination occurs in the unsensitized latex control. Bactigen uses a special buffer which is claimed to minimize nonspecific reactions.

All the products offer advantages to the clinical microbiology laboratory and enable rapid, sensitive, and specific detection of the four major agents of bacterial meningitis. The superiority of these kits over CIE is clear, and they can effectively replace CIE in most laboratories.

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