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Evaluation of the Phadebact CSF Test for Detection of the Four Most Common Causes of Bacterial Meningitis

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A five-center collaborative study was undertaken to determine the suitability of the Phadebact CSF test kit and the Phadebact group B *Streptococcus* reagent for routine use by clinical laboratories to detect antigens of common organisms causing bacterial meningitis. The kits employ staphylococcal protein A coagglutination to detect the antigens of *Haemophilus influenzae* types a, b, c, d, e, and f, *Neisseria meningitidis* groups A, B, C, Y, and W135, *Streptococcus pneumoniae* (83 serotypes), and group B *Streptococcus*. A total of 2,817 individual tests were performed on 577 cerebrospinal fluid specimens. The percent positive specimens detected by coagglutination was as follows: overall, 84%; *H. influenzae*, 97%; group B *Streptococcus*, 75%; *S. pneumoniae*, 71%; and *N. meningitidis*, 58%. Eighty-five of the specimens were also tested by counterimmunoelectrophoresis. Coagglutination was more sensitive than counterimmunoelectrophoresis because it detected 74% of the positive specimens, whereas counterimmunoelectrophoresis detected only 65%. No false-positive results were obtained with coagglutination. The Phadebact CSF test kit is recommended for routine use in screening cerebrospinal fluid samples for antigens of the common organisms causing bacterial meningitis along with the Gram stain and culture for delayed confirmation of the rapid results.

Over the past 10 years there has been increasing interest in the development of rapid (1 h or less) reliable methods for detection of agents of bacterial meningitis. Three of the most promising techniques have been counterimmunoelectrophoresis (CIEP), originally described by Bussard in 1959 (3), latex agglutination, and staphylococcal protein A coagglutination (COA). Newman et al. (7) first utilized latex agglutination in 1970 for the diagnosis of meningitis caused by *Haemophilus influenzae*, and in 1973 Kronvall (6) introduced the COA procedure for typing pneumococci. Since then numerous reports on these three procedures have appeared for detection of antigens in cerebrospinal fluid (CSF). There is still no general agreement as to the value and place of these methods in the routine clinical microbiology laboratory for detection of the most common bacterial agents causing meningitis, namely, *H. influenzae*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, and group B *Streptococcus*. There does, however, appear to be agreement on two disadvantages of CIEP, the need for equipment not normally found in clinical microbiology laboratories and insufficient sensitivity, particularly in the case of *S. pneumoniae*. These problems were reconfirmed recently by Wasilauskas and Hampton (10). They compared CIEP with COA and culture and found COA to be more sensitive than CIEP in detection of bacterial meningitis. In another recent study by Welch and Hensel (12) comparing latex agglutination and COA with cultures for detection of *H. influenzae* type b meningitis in CSF, they found latex agglutination and COA gave similar results, and both were more sensitive than CIEP.

To assess the value of the rapid COA technique for routine use in clinical laboratories, we report here the results of a collaborative study involving five centers in which 2,817 individual tests were performed on 577 spinal fluid specimens from both adult and pediatric patients with the Phadebact CSF test kit. The kit contains reagents for detection of antigens of *H. influenzae* b, *H. influenzae* a, c, d, and e, *S. pneumo-*

niae, and *N. meningitidis* in CSF. Individual reagents were used for detection of group B *Streptococcus*.

MATERIALS AND METHODS

Collaborative study. A protocol was agreed upon by the collaborating investigators. Each participating center tested CSF from patients with the Phadebact CSF test kit or individual Phadebact COA reagents in addition to their regular procedures for handling CSF. Both banked, frozen and fresh clinical specimens were used. At centers in which CIEP was in routine use, some of the specimens were also tested by CIEP for comparison with COA.

Bacteriological cultures. Each participating center used standard methods approved by the College of American Pathologists for the isolation and identification of CSF isolates.

COA tests. Phadebact CSF test kits and individual COA reagents were provided by Pharmacia Diagnostics (Piscataway, N.J.). All CSF specimens, both culture positive and culture negative, were tested with each of the following reagents: *H. influenzae* type b; *H. influenzae* types a, c, d, e, and f; *S. pneumoniae*, consisting of 83 serotypes; and *N. meningitidis* groups A, B, C, Y, and W135. Some of the specimens were also tested with the Phadebact group B *Streptococcus* reagent. The reagents were ready to use and contained methylene blue for easy visualization of results. Before testing, each specimen was heated at 80°C for 5 min to eliminate nonspecific reactions. If the specimen was grossly cloudy or bloody, it was centrifuged before heating. Heat-treated CSF (1 drop) was mixed with 1 drop of each test reagent on a disposable, white card. The card slide was rocked manually for 1 to 2 min. COA results were recorded as positive if a significantly stronger and more rapid reaction occurred with one reagent compared with the other three reagents being tested on the slide at the same time. Results were considered negative if no agglutination was observed with any of the test reagents. Results were considered to be noninterpretable if COA occurred at equally strong intensity and speed with more than one reagent. Quality control procedures were performed according to the instructions of the manufacturer. Positive control reagents were prepared by heating saline suspensions (10^8 organisms per ml) of the respective laboratory-confirmed strains at 80°C for 5 min. These suspensions were tested as the clinical specimens. The simultaneous use of four reagents in testing an unknown specimen was considered a built-in negative control.

CIEP testing. CIEP was performed by standard methods and with reagents which have been detailed elsewhere (1, 4, 12).

Evaluation of results. CSF specimens were presumed to contain specific bacterial antigens if any one of the following criteria was met: (i) a positive culture was obtained, (ii) a positive COA test was obtained from a patient with bacteremia due to the same organism as detected by COA in the CSF, or (iii) a positive COA test was obtained on a CSF specimen subsequent to an earlier test of a patient that was culture positive, indicating persistence of antigen during antibiotic therapy.

TABLE 1. COA results with positive CSF specimens[a]

Center[b]	*H. influenzae* b			*S. pneumoniae*			*N. meningitidis*			Group B *Streptococcus*		
	COA positive	COA negative	Sensitivity (%)	COA positive	COA negative	Sensitivity (%)	COA positive	COA negative	Sensitivity (%)	COA positive	COA negative	Sensitivity (%)
TTU	22	0	100	12[c]	0	100	7	4	64	2	2	50
OCMH	62	0	100	12	6	67	7	5	64	4	0	100
ACH	16	4	80	2	3	40	0	4	0	1	0	100
UTGAL	12	0	100	4	2	75	3	1	75			
ALG	6	0	100	2	2	50	2	0	100			

[a] Positive specimens include those that were culture positive as well as specimens taken postantibiotic therapy. Initial specimen was culture positive. Overall sensitivity was 84% (true positives/true positives + false negatives × 100).

[b] TTU, Texas Tech Regional Academic Health Center at El Paso, Tex.; OCMH, University of Oklahoma Health Sciences Center, Oklahoma City, Okla.; ACH, Arkansas Children's Hospital, Little Rock, Ark.; UTGAL, The University of Texas at Galveston, Galveston, Tex.; ALG, Allegheny General Hospital, Pittsburgh, Pa.

[c] Test results using single COA reagent, not CSF kit reagent.

RESULTS

Of the 577 CSF specimens included in the study, 209 were positive by the criteria previously described for the test organisms (*H. influenzae* types a, b, c, d, e, or f; *S. pneumoniae*, 83 serotypes; *N. meningitidis* groups A, B, C, Y, or W135; and group B *Streptococcus*), 344 were culture negative, and 24 were culture positive for organisms other than test organisms. Table 1 summarizes the COA results obtained on the positive specimens. Of the 209 positive specimens, 176 were positive by COA for an overall sensitivity of 84%. There were no false positives, therefore the specificity was 100%. There was good agreement among participants on detection of *H. influenzae* b. Of 122 specimens, 118 (97%) were positive by COA. Five of the COA-positive specimens were collected postantibiotic therapy. However, the rate of detection of *S. pneumoniae* was variable. Of 45 specimens, 32 were positive by COA, but detection rates among the participants ranged from 40 to 100%. Investigators at Texas Tech University, the center with high correlation between culture and COA results, used the individual pneumococcal reagent, not the reagent supplied with the CSF kit, to test their 12 pneumococcus culture-positive CSF specimens. Six of the CSF specimens still available were retested with the material provided with the CSF kit. One had a strongly positive (4+) reaction, two were weakly positive (±), and three were negative (−) for a 50% sensitivity instead of the 100% obtained with the individual pneumococcal reagent. The new results more closely agreed with those obtained at the other centers. Of 33 culture-positive specimens for *N. meningitidis*, 19 were positive by COA for an overall sensitivity of 58%. Seven of nine specimens were positive for group B *Streptococcus* for 78% sensitivity. There were no false positives since none of the 344 CSF specimens that were culture negative were positive for any of the screen antigens. Table 2 gives COA results obtained with culture-positive specimens for organisms other than the screen organisms. None of 14 different organisms from a total of 24 specimens were COA positive.

TABLE 2. COA results with culture-positive CSF specimens for organisms other than the screen organisms

Organism	No. of specimens COA negative[a]
Escherichia coli	5
Staphylococcus aureus	3
Enterococcus sp.	2
Group A Streptococcus	2
Klebsiella pneumoniae	2
Listeria monocytogenes	2
Cryptococcus neoformans	1
Bacillus sp.	1
Staphylococcus epidermidis	1
Haemophilus influenzae a[b]	1
Haemophilus influenzae f[b]	1
Mycobacterium tuberculosis	1
Proteus mirabilis	1
Moraxella morganii	1

[a] Each specimen was tested with *H. influenzae* b, *S. pneumoniae*, *N. meningitidis*, and group B *Streptococcus* COA reagents. No specimens were COA positive with these reagents, except as noted.

[b] Positive with the *H. influenza* a, c, d, e, and f reagents.

TABLE 3. Comparison of COA and CIEP for detection of antigen in culture-positive clinical specimens

Organism isolated	No. of specimens[a]			
	COA positive	COA negative	CIEP positive	CIEP negative
H. influenzae b	34	1	31	4
S. pneumoniae	16	12	16	12
N. meningitidis	13	9	8	14

[a] Total COA positive, 74%; COA negative, 26%; CIEP positive, 65%; and CIEP negative, 35%.

CIEP was run on 85 of the culture-positive specimens, and the results are given in Table 3. A total of 63 (74%) were positive by COA, and 55 (65%) were positive by CIEP. *S. pneumoniae* was detected with equal sensitivity by COA and CIEP, but COA was better in detecting *H. influenzae* b and *N. meningitidis*. Of the 33 positive *N. meningitidis* specimens included in the study, serotypes were known for 16 of them. Nine were group B, six were group C, and one was W135. Of group B, three of nine specimens were positive as were four of six of group C; the one W135 specimen was negative.

DISCUSSION

Our results with 118 positive *H. influenzae* b CSF specimens conclusively confirms the findings of previous smaller studies which indicated that COA is an accurate and sensitive method for the rapid detection of *H. influenzae* b. This is significant because 67% of bacterial meningitis in children under 10 years of age is caused by *H. influenzae* b (2). The ability of COA to detect the continuing presence of antigen when cultures are negative should prove helpful in the clinical management of patients, as was shown with five culture-negative specimens that were COA positive for *H. influenzae* b. An advantage of the Phadebact kit is that it detects not only type b, but also types a, c, d, e, and f, which occasional-

ly cause meningitis. One type a and one type f were included in our study, and both were positive by COA. The nontype b reagents also serve as a good negative control for the assurance of specificity.

The detection of *S. pneumoniae* in positive specimens by participants in this study varied between 40 and 100%. Since investigators at the center with the highest correlation with culture results used the individual pneumococcal reagent, marketed for the identification of isolates, as did Wasilauskas and Hampton (10) who also had a high correlation with culture results, it is possible that a difference in the reagents accounts for the discrepancy in results. The manufacturer is reviewing the assay kinetics with the intent of improving the pneumococcal CSF reagent.

Some of the culture-positive, COA-negative results may have been due to the numbers of organisms present or the antigen concentration in the clinical specimens, which may have been below the detection limit for COA. Ölcen (8) found that he could not detect concentrations below 10^5 CFU/ml, whereas Wetkowski et al. (13) found that 10^7 to 10^8 CFU/ml were necessary for detection of streptococci in positive blood cultures. Colony counts were known for two of the *N. meningitidis* specimens; one specimen did not grow on solid media and the organism was recovered only in broth, the other produced 120 colonies or ca. 120,000 CFU/ml, which is at or below the detection limit reported in the above-mentioned studies.

Although seven of nine (78%) group B *Streptococcus* specimens were positive by COA, more specimens need to be tested before the detection efficiency can be determined accurately. The detection rates of group B *Streptococcus* in two previous studies were 87% by Wasilauskas and Hampton (10) with 8 specimens and 83% by Webb et al. (11) with 23 specimens.

Of the 77 specimens tested by both COA and CIEP, 74% were positive by COA and 65% were positive by CIEP. We conclude, as have others (5, 9, 11), that COA is more sensitive.

In summary, COA is easy to perform and fast, requiring 30 min or less as opposed 1.5 to 2 h for CIEP. The cost of screening a CSF specimen with the Phadebact CSF text kit is approximately $10.50 based on a reagent cost of $9.00 and a labor cost of $10.00 per h. Although the Gram stain is a rapid diagnostic method currently available, it lacks the sensitivity of COA. COA and the Gram stain should be used in conjunction with cultures for the delayed confirmation of the rapid results and for antibiotic sensitivity testing of the organisms. The future of clinically relevant diagnostic microbiology rests with the development of rapid, sensitive, and specific tests for antigen detection. Techniques like COA will continue to gain in popularity because they are simple and rapid.

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