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Detection of Bacterial Antigens in Body Fluids with the Wellcogen *Haemophilus influenzae* b, *Streptococcus pneumoniae*, and *Neisseria meningitidis* (ACYW135) Latex Agglutination Tests

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The Wellcogen *Haemophilus influenzae* b, *Streptococcus pneumoniae*, and *Neisseria meningitidis* (ACYW135) latex agglutination tests (Wellcome Diagnostics, Dartford, England) were evaluated as methods to detect bacterial antigens in cerebrospinal fluid (CSF), urine, and serum from patients with meningitis or sepsis. Antigen was detected in 92% of CSFs from *H. influenzae* b, 100% of CSFs from *N. meningitidis* groups A and Y, 36% of CSFs from *N. meningitidis* group C, and 69% of CSFs from pneumococcal meningitis patients. Serum samples presented a problem, with a few false-positive or possible cross-reactions. The Wellcogen latex agglutination tests were more sensitive than the Bactogen (*H. influenzae* type b) latex agglutination test and the Phadebact (*S. pneumoniae*) co-agglutination test.

The purpose of this study was to evaluate the Wellcogen *Haemophilus influenzae* b, *Streptococcus pneumoniae*, and *Neisseria meningitidis* (ACYW135) latex agglutination tests (Wellcome Diagnostics, Dartford, England) as methods to detect *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis* groups A, C, and Y antigens in body fluids of patients with systemic disease (meningitis or sepsis or both). These reagents were compared in their sensitivity with the Bactogen latex slide test (Wampole Laboratories, Cranbury, N.J.) for *H. influenzae* type b, with the Phadebact co-agglutination pneumococcus test (Pharmacia Diagnostics, Uppsala, Sweden), and with countercurrent immunoelectrophoresis (CIE).

MATERIALS AND METHODS

The Wellcogen *H. influenzae* b, *S. pneumoniae*, and *N. meningitidis* (ACYW135) latex agglutination tests were performed by mixing 20 μl of the latex test or control reagent with 40 μl of cerebrospinal fluid (CSF), urine, or serum being tested for antigen on a glass slide and then rocking the slide for 3 min. The reaction was recorded as positive when clumping of the latex suspension was clearly visible. When noted, urine samples were concentrated 10 or more times in a disposable ultrafilter system (Minicon B-15) filter (Amicon Corp., Lexington, Mass.). Serum that agglutinated the negative control latex was heated at 56°C for 30 min, cooled, and centrifuged at 1,000 × *g* for 5 min, and the supernatant was retested.

Body fluids from patients with *H. influenzae* type b systemic disease were also tested by using the Bactogen latex agglutination slide test (Wampole, lot no. 1K716N) according to the manufacturer's instructions. CSF samples from patients with pneumococcal meningitis were tested by using the Phadebact co-agglutination test (Pharmacia lot no. 3959) according to the manufacturer's instructions.

CIE was performed by using 1% Noble agar plus agarose in 0.05 M sodium barbital buffer (pH 8.6) with 0.05 M sodium barbital (pH 8.6) as the liquid buffer. A 10-μl body fluid sample was placed in the cathode well, and 10 μl of antiserum was placed in the anode well. Plates were electrophoresed for 60 min at 30 mA, using a Hyland power pack system, and were observed for precipitin lines. Samples were electrophoresed against antiserum to the organism obtained on culture from the sample. Antisera used were: *H. influenzae* type b antiserum (Burro no. 132), *N. meningitidis* group C antiserum (Burro no. 211 and 178) from J. Robbins, Bureau of Biologics, Rockville, Md., *S. pneumoniae* omni sera from Statens Seruminstitut, Copenhagen, Denmark, and *N. meningitidis* group A antiserum (Wellcome). The sensitivity of the CIE system in detecting purified capsular antigen was 25 ng/ml for *H. influenzae* type b, 500 ng/ml for *N. meningitidis* group A, 50 ng/ml for *N. meningitidis* group C, and between 500 and 5,000 ng/ml for *S. pneumoniae* types 3, 6, 9, 14, 19, and 23.

The CSF samples tested were obtained from patients with bacterial culture-proven *H. influenzae* type b, *S. pneumoniae*, *N. meningitidis* group A, B, C, or Y, *Escherichia coli*, or aseptic meningitis. Serum and urine samples were obtained from patients with posi-

TABLE 1. Results of testing

Body fluid (no. of samples)[a]	No. of positive reactions with the following test (%)[b]:						
	Wellcogen			Bactogen	Phadebact	CIE	Gram stain
	Hb	Pn	Mc	Hb	Pn		
Hb CSF (39)	36 (92)	0	0	32 (82)	NT	32 (82)	35/38 (92)
Hb serum (12)	11 (92)	0	0	7 (58)	NT	4 (33)	NT
Hb urine (3)	3 (100)	0	0	3 (100)	NT	3 (100)	NT
Pn CSF (16)	0	11 (69)	0	NT	4 (25)	9 (56)	12/14 (86)
Pn serum (15)	2[c]	1 (7)	1[c]	NT	NT	0	NT
Pn urine (5)	0	0	0	NT	NT	1 (20)	NT
Mc CSF (5) (group A)	0	0	5	NT	NT	5 (100)	NT
Mc CSF (2) (group B)	0	0	0	NT	NT	NT	1 (50)
Mc serum (1) (group B)	0	0	0	NT	NT	NT	NT
Mc urine (1) (group B)	0	0	0	NT	NT	NT	NT
Mc CSF (11) (group C)	0	0	4 (36)	NT	NT	0	8/9 (89)
Mc serum (5) (group C)	1[c]	0	3 (60)	NT	NT	2 (40)	NT
Mc CSF (1) (group Y)	0	0	1 (100)	NT	NT	1 (100)	1 (100)
E. coli CSF (4)	0	0	0	NT	NT	NT	3 (75)
Aseptic CSF (10)	0	0	0	NT	NT	NT	0 (0)

[a] Hb, *H. influenzae* type b; Pn, *S. pneumoniae*; Mc, *N. meningitidis*.
[b] NT, Not tested.
[c] False-positive reaction(s).

tive blood or CSF cultures for bacteria. Body fluids were collected at the Wake County Medical Center, Raleigh, N.C., between January 1974 and September 1982. All samples were centrifuged for 2.5 min at 3,200 × *g*, and the supernatant was stored at −20°C until tested. *N. meningitidis* group A CSFs were obtained from the Centers for Disease Control, Atlanta, Ga.

Samples had been cultured for bacteria by routine methods. Typing or grouping of isolates was performed at the North Carolina State Laboratory of Public Health, Raleigh, N.C. or the Centers for Disease Control, Atlanta, Ga.

Many of these samples had had the antigen concentration determined by latex agglutination or CIE prospectively, and the antigen titers were rechecked to determine whether they had changed during storage. Prospective Gram stain results on the CSF samples were recorded.

The sensitivity, specificity, and positive and negative predictive values were determined for the Wellcogen tests.

Sensitivity is defined as the ability of the test to identify correctly all of the diseased patients as diseased. Specificity is defined as the ability of the test to identify correctly all of the nondiseased patients as nondiseased.

The positive predictive value is the percentage of positive test results that were positive in diseased patients. The negative predictive value is the percentage of negative test results that were negative in nondiseased patients.

RESULTS

The number of various types of body fluids and the number of cross-reactions or false-positive reactions are shown in Table 1. By the use of the Wellcogen *H. influenzae* b test, antigen was detected in 92% of CSFs from patients with *H. influenzae* b meningitis and 92% of serum samples and 100% of three urine samples tested from patients with *H. influenzae* b systemic disease. The Bactogen test on the samples was less sensitive. With the Wellcogen *S. pneumoniae* test, pneumococcal antigen was detected in 69% of CSFs from patients with pneumococcal meningitis, in 7% of serum samples, and in none of the five concentrated urine samples from patients with systemic pneumococcal disease. The Phadebact test was less sensitive in detecting pneumococcal antigen in CSFs of patients with pneumococcal meningitis.

The Wellcogen *N. meningitidis* (ACYW135) test detected antigen in 36% of CSFs from patients with *N. meningitidis* group C meningitis, in all patients with *N. meningitidis* groups A and Y meningitis, and in 60% of the serum samples from patients with *N. meningitidis* group C disease. The Wellcogen reagents were more sensitive than CIE, except for one pneumococcal urine sample and two pneumococcal CSFs in which antigen was detected by CIE alone. Sensitivity, specificity, and positive and negative predictive values for the Wellcogen reagents are shown in Table 2.

No nonspecific or false-positive reactions were noted when CSF or urine was tested. There were four false-positive tests (Table 1) when serum samples were tested with the Wellcogen reagents. These reactions were not removed by heating the samples at 56°C for 30 min.

Retrospective testing of frozen samples revealed no loss of antigen as compared with the prospective testing by CIE or latex agglutination testing up to 8 years previously.

TABLE 2. Wellcogen reagents: sensitivity, specificity, and positive and negative predictive values

Body fluid (no. of samples) and Wellcogen reagent[a]	% Positive reactions			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
CSF (88)				
Hb	92	100	100	94
Pn	69	100	100	94
Mc	59	100	100	91
Serum (33)				
Hb	92	86	79	95
Pn	7	100	100	56
Mc	60	96	75	93
Urine (9)				
Hb	100	100	100	100
Pn	0	100	0	44
Mc	—	—	—	—

[a] Abbreviations are the same as in Table 1.

DISCUSSION

As latex agglutination tests for detecting bacterial antigens are becoming commercially available, it is important to evaluate their sensitivity, specificity, and predictive values so that recommendations may be made concerning their usefulness. By the use of the Wellcogen *H. influenzae* b test, our study has shown that antigen can be detected in a high percentage of CSF, serum, and urine samples from patients with systemic *H. influenzae* type b disease. The Wellcogen *H. influenzae* b test was more sensitive than the Bactogen test. The Wellcogen reagents detected antigen in all of the *N. meningitidis* group A and Y patients. A smaller percentage of patients with systemic disease caused by *S. pneumoniae* and *N. meningitidis* group C had detectable bacterial antigen in body fluids. The Phadebact test was less sensitive than the Wellcogen *S. pneumoniae* latex agglutination test. Lower rates of detection of bacterial antigens in body fluids of patients with *S. pneumoniae* and *N. meningitidis* group C systemic disease than in those with *H. influenzae* type b have been noted by others when noncommercially available latex agglutination tests were used (2).

Rare cross-reactions between the capsular polysaccharides of various species of bacteria are noted in the literature on the use of latex agglutination tests and other antigen detection systems (1, 2). In our study, there were four reactions due to true cross-reactions between species of bacteria or to serum components that were not removed by heating at 56°C. Whether or not these were true cross-reactions could not be determined, as the organisms isolated from these patients were not available for testing. Nonspecific reactions in CSF and urine often can be removed by boiling, cooling, centrifugation, and retesting of the body fluid. Wellcome Diagnostics now recommends heating the serum sample for 5 min in a boiling water bath after the addition of 3 volumes of 0.1 M EDTA, but this procedure was not used in this study. There is also a concern that *N. meningitidis* but not *H. influenzae* type b or *S. pneumoniae* antigen may be heat sensitive at 100°C for 5 min (3). *N. meningitidis* group A antigens—but not those of groups C or Y—detected by the latex agglutination test become undetectable when heated at 100°C for 5 min with one lot but not a second lot of the Wellcogen test. Heating did not affect antigens detected by CIE (D. L. Ingram and A. R. Occhiuti, unpublished data).

Despite rare problems with true cross-reactions and problems with removing false-positive reactions from serum, a positive result with the Wellcogen latex agglutination tests for the antigens studied can be useful information. Because of the speed (3 min) and simplicity of the latex agglutination tests, a tentative diagnosis can often be made quickly. As with all tests, the positive results must be put in the context of the other clinical and laboratory findings to determine the diagnosis of a patient. As noted in this study, the Gram stain of the CSF may be more sensitive than the latex agglutination tests and should always be performed. Manufacturers' instructions concerning the processing and testing of specimens must be followed carefully to avoid false-positive reactions that could lead to unnecessary treatment of patients.

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