JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1986, p. 440–445
0095-1137/86/090440-06$02.00/0

Vol. 24, No. 3

Rapid Diagnosis of Severe *Haemophilus influenzae* Serotype b Infections by Monoclonal Antibody Enzyme Immunoassay for Outer Membrane Proteins

ABDELLAH BELMAAZA,[1] JOSÉE HAMEL,[1] SERGE MOUSSEAU,[1] SERGE MONTPLAISIR,[1]* AND BERNARD R. BRODEUR[2]

Department of Microbiology and Immunology, Université de Montréal and Hôpital Sainte-Justine, Centre de Recherche Pédiatrique, Montreal,[1] and Hybridoma Section, Bureau of Microbiology, Laboratory Center for Disease Control, Health and Welfare, Ottawa,[2] Canada

Received 23 January 1986/Accepted 11 June 1986

A highly sensitive and specific enzyme immunoassay (EIA) for the detection of *Haemophilus influenzae* serotype b antigens in body fluids and broth cultures was developed, with a polyclonal antibody directed against polyribose phosphate as the solid-phase reagent and a biotinylated monoclonal antibody directed against *H. influenzae* type b outer membrane protein as the liquid-phase reagent. *H. influenzae* type b antigens could be detected in broth cultures containing as little as 50 organisms per ml. The sensitivity and specificity of this system were compared with those of two commercial kits and counterimmunoelectrophoresis. The overall detection of *H. influenzae* type b antigens in clinical specimens collected from children infected with *H. influenzae* type b was as follows: with Phadebact, 86 and 86% in cerebrospinal fluid and urine specimens, respectively; with Bactigen, 86, 80, and 92%, with counterimmunoelectrophoresis, 78, 73, and 75%, and with biotin-avidin EIA, 100, 100, and 100% for cerebrospinal fluid, serum, and urine specimens, respectively. In the biotin-avidin EIA, no positive reactions were noted in specimens collected from patients infected with other bacteria or from patients without evidence of bacterial infection, whereas false-positive reactions were found by counterimmunoelectrophoresis and the commercial kits. These results suggest that this monoclonal antibody reacting with the outer membrane protein is more specific and sensitive than the conventional methods using polyclonal antisera for the detection of *H. influenzae* type b antigens during severe infections in children.

The rapid detection of soluble bacterial antigens in the body fluids of patients presenting with severe infections has distinct advantages. The result of the antigen detection test is available at least 18 h before the result of the culture and is especially useful when Gram staining and culture remain negative.

A variety of tests have already been used for the detection of bacterial antigens. These include counterimmunoelectrophoresis (CIE) (7, 13, 14, 18, 20), latex agglutination (LA) (10, 21, 24, 28, 30), coagglutination (COA) (10, 26, 27, 29, 30), and enzyme-linked immunosorbent assay (8, 11, 15, 19, 25). The immunodiagnostic reagents used for the detection of *Haemophilus influenzae* serotype b antigen (polyribose phosphate [PRP]) show false-positive results with *Escherichia coli*, *Streptococcus pneumoniae*, and other bacteria (3, 9, 12, 13, 19, 23, 25). Most of these cross-reactions are due to the nonspecificity of the polyclonal antisera used. Some contaminants may persist in the extract used as an immunogen, despite rigorous purification. Alternatively, preexisting antibody from previous natural infection may be present in the serum of the hyperimmunized animal and may give rise to false-positive reactions (31). A new era in serology was promoted by hybridoma technology (17), from which monoclonal antibodies (MAb) are produced and can reduce to a minimal level the incidence of cross-reactivity among bacterial antigens and consequently increase the specificity of these tests.

In the present study, we describe the detection of bacterial antigens, namely PRP and outer membrane proteins (OMPs), in the body fluids collected from children presenting with a severe infection caused by *H. influenzae* serotype b. An enzyme immunoassay using both rabbit polyclonal and murine MAbs permitted comparison of the sensitivity and specificity of this new assay with those of three other methods, including the LA test, LA-Bactigen; the COA test, COA-Phadebact; and CIE.

MATERIALS AND METHODS

Clinical specimens and bacterial strains. Cerebrospinal fluid (CSF), serum, and urine specimens were collected from children admitted to Sainte-Justine Hospital (Montreal, Canada) from January 1984 to February 1985 with a proven diagnosis of *H. influenzae* serotype b infection, as detected by culture or in two different serological tests. The specimens were processed for Gram staining and culture on chocolate agar plates. When not subjected to antigen testing immediately, the specimens were kept frozen at −70°C.

H. influenzae capsular serotypes a, b, d, and e and untypable *H. influenzae* strains were obtained from clinical isolates from the following Canadian hospitals: Sainte-Justine Hospital, Montreal Children's Hospital, Vancouver Children's Hospital, Children's Hospital of Eastern Ontario, and Laboratoire de la Santé Publique du Québec. *H. influenzae* strains were identified by their requirements for both X and V factors, and capsular serotype was determined by slide agglutination with commercially available serum (Difco Laboratories, Detroit, Mich.). *H. influenzae* was routinely grown overnight at 37°C, in an atmosphere containing 5% CO_2, on chocolate agar plates supplemented with 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.). Cultures were stored at −70°C in brain heart infusion

* Corresponding author.

broth containing 20% glycerol or were kept lyophilized in skim milk.

MAb production and purification. BALB/c mice were injected intraperitoneally and in the foot pads with an extract of 50 μg of OMPs (4) from *H. influenzae* serotype b mixed with Freund incomplete adjuvant. Four weeks later, mice were immunized intravenously with 30 μg of OMPs suspended in phosphate-buffered saline (PBS). Four days before hybridoma production, the mice received the last intravenous injection of 30 μg of OMPs. MAbs against OMP were obtained by fusing sensitized spleen cells with the nonsecreting mouse myeloma SP2/0 cells as previously described (5). The antibody-producing cells were cloned twice by limiting dilution, and ascitic fluids were produced in mice by the procedure described by Brodeur et al. (6). Antibody class and subclass were determined by double radial immunodiffusion. Culture supernatants were concentrated 10 times by ammonium sulfate precipitation and were tested against rabbit anti-mouse immunoglobulin G1 (IgG1), IgG2a, IgG2b, IgG3, and IgM(μ) heavy-chain antisera (Meloy, Springfield, Va.).

Ascitic fluids containing MAb Hb_1 were passed through glass wool and treated with 20 mg of Aerosil 380 (Degussa, Federal Republic of Germany) per ml of ascitic fluids to remove the β-lipoproteins. The mixture was stirred for 4 h at room temperature, and the precipitate was removed by centrifugation at 12,000 × *g* for 30 min. The treated ascitic fluids were applied on Affi-Gel–protein A (Bio-Rad Laboratories, Richmond, Calif.). The concentration of the IgG proteins was determined spectrophotometrically at 280 nm.

Polyclonal rabbit antiserum directed against PRP was purchased from the New York State Department of Health, Division of Laboratories and Research, Albany, N.Y.

Biotinylation procedure. Biotin was covalently conjugated to a purified preparation of MAb Hb_1 and normal mouse IgG by a modification of the method described by Bayer and Wilcheck (2). Briefly, biotin (BIO-CAP-NHS; Calbiochem-Behring; La Jolla, Calif.) was dissolved in dimethylformamide at various concentrations. A 100-μl volume of each dilution was added to 1 ml of IgG diluted to a concentration of 3 mg/ml in PBS at pH 7.2. The reaction mixture was rocked for 3 h at 25°C and dialyzed at 4°C against two changes of PBS. After dialysis, an equal volume of glycerol was added, and the biotinylated MAb and normal mouse IgG preparations were stored at −20°C.

B-A EIAs. All biotin-avidin enzyme immunoassays (B-A EIAs) were performed in rigid, nonsterile, flat-bottom microtiter plates (Immulon II; Dynatech Laboratories, Inc., Alexandria, Va.). Wells were coated overnight at room temperature with 200 μl of rabbit anti-PRP (1 μg/ml) suspended in 0.1 M sodium bicarbonate (pH 9.6) and postcoated with 300 μl of PBS-Tween 20 (0.05%) containing 2% bovine serum albumin and 1% L-lysine for 30 min at 37°C. The plates were kept at −20°C until used. Before use, the coated plates were washed three times with PBS-Tween 20. Clinical specimens of CSF, serum, and urine were diluted 1:2 in PBS-Tween 20, and a 200-μl volume was added to each well. The plates were incubated for 2 h at 37°C, followed by three washes with 250 μl of PBS-Tween 20. Two wells of each specimen were treated with biotinylated normal mouse IgG, and two were treated with biotinylated MAb (2 μg/ml). After a 1-h incubation at 37°C, the plates were washed three times, a 200-μl volume of avidin-peroxidase (Sigma Chemical Co., St. Louis, Mo.) diluted 1:1,000 was added, and the plates were incubated for 20 min at room temperature and then washed. A 200-μl volume of 5-aminosalicylic acid (80 mg dissolved in distilled water at 70°C, cooled to 25°C, and adjusted to pH 6 with NaOH) containing 0.0025% H_2O_2 was added. After 30 min at room temperature, the A_{490} for each well was determined with a micro-ELISA reader (MR 600; Dynatech). The test was considered to be positive if the A_{490} was three standard deviations greater than that of the negative control (clinical specimens collected from patients who had no well-documented previous exposure to *H. influenzae* type b).

LA test. The LA test (Bactigen; Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N.J.) was used to test CSF, serum, and urine specimens. These samples were tested according to the instructions of the manufacturer, with the *Haemophilus* antibody latex and control latex reagents. The CSF specimens were not heat treated, but cloudy or bloody specimens were centrifuged for 10 min at 1,000 × *g* before testing.

COA test. The COA *Haemophilus* test (Phadebact) was purchased from Pharmacia (Uppsala, Sweden). All of the CSF specimens, from both positive and negative cultures, were tested with each of the following reagents: *H. influenzae* serotype b; *H. influenzae* serotypes a, c, d, e, and f; *S. pneumoniae*; and group B *Streptococcus* sp. Before testing, each specimen was heated at 80°C for 5 min to eliminate nonspecific reactions. COA results were considered to be positive if a significantly stronger and more rapid reaction occurred with any of the test slides at the same time. Results were considered to be negative if no COA was observed with any of the test reagents. The results were considered to be noninterpretable if the COA occurred at equal intensity and speed with more than one reagent.

CIE. CIE was performed with *H. influenzae* type b antiserum (Statens Seruminstitut, Copenhagen, Denmark). Clinical specimens (CSF, serum, and urine) and antiserum were placed in 5-mm wells spaced 3 mm apart on a 1% agarose-coated glass slide. The slides were electrophoresed in a barbital buffer (pH 8.6) for 90 min at 30 mA. The presence of a sharp precipitin band was considered to be a positive test. Plates showing broad precipitin bands were soaked in normal saline overnight and observed again the next day (20).

The statistical test used for comparison of the B-A EIA, CIE, COA, and LA methods was Cochran's test for the comparison of percentages in matched samples (30a).

RESULTS

Properties of the MAb. MAb Hb_1 was screened for different *H. influenzae* strains by a dot-enzyme immunoassay performed as described previously (5). Hb_1 reacted specifically with 276 strains of *H. influenzae* type b but not with the other capsular serotype or untypable strains. The characterization of Hb_1 (IgG1) by Western immunoblotting analysis revealed that it is directed against the 37,000-dalton OMP (Fig. 1). In addition, Hb_1 was shown to be directed towards an epitope exposed on the cell surface, as demonstrated by a competition test using a dot-enzyme immunoassay (J. Hamel, B. R. Brodeur, and S. Montplaisir, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, E56, p. 105).

Sensitivity. The optimal binding ratio of biotin to MAb Hb_1 that yielded the highest sensitivity in B-A EIA was found to be 1:3. The sensitivity of the B-A EIA was evaluated by using serial dilution of *H. influenzae* serotype b organisms. The minimum number of bacteria giving a positive reaction with an absorbance value of 0.095 was 50 bacteria per ml (Fig. 2).

Specificity. To evaluate the specificity of MAb Hb_1 used in the B-A EIA, we tested a panel of microorganisms (10^5

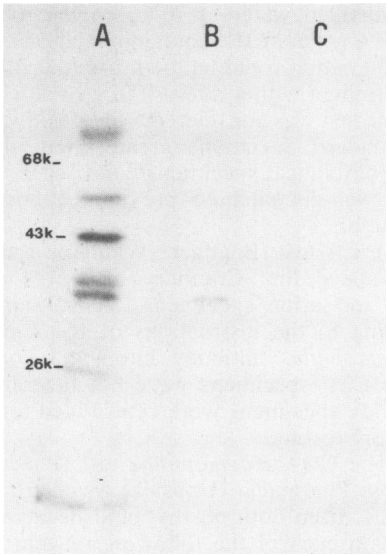

FIG. 1. Detection of MAb Hb_1 directed against OMP of *H. influenzae* type b by immunoblotting. OMPs were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After electrophoretic transfer, the blots were treated with 5% (wt/vol) skim milk and then incubated with mouse hyperimmune serum (A), MAb Hb_1 (B), or SP2/0-induced ascitic fluids diluted in skim milk (C). Specific OMPs were revealed by the use of ^{125}I-labeled anti-mouse serum autoradiography.

CFU/ml) isolated from children infected with one of a variety of bacterial strains: *Staphylococcus* sp. (17 cases), *S. pneumoniae* (12 cases), *Streptococcus pyogenes* (5 cases), group B *Streptococcus* sp. (17 cases), *Neisseria meningitidis* (11 cases), *E. coli* (9 cases), *H. influenzae* capsular serotype a, d, or e (15 cases), and *Haemophilus parainfluenzae* (10 cases). None of these bacteria gave an A_{490} greater than 0.061 (Table 1).

CSF. A total of 18 CSF specimens collected from 18 children presenting with meningitis were tested by CIE, two commercial kits (LA-Bactigen and COA-Phadebact), and B-A EIA. Among these samples, 12 were positive and 6 were negative by culture; the 6 culture-negative CSF specimens had a characteristic Gram stain, and *H. influenzae* serotype b antigens were detected in more than two serological tests. A comparative study by these four different methods revealed that the B-A EIA was the most sensitive one for both groups (Table 2). The differences in sensitivity between B-A EIA and CIE, B-A EIA and COA, and B-A EIA and LA were significant, with P values of 0.025, 0.083, and 0.083, respectively. Among the CSF-positive culture group, we found false-negative results which are represented by four patients, the first two being negative by CIE, COA, and LA and the third being negative by LA only, whereas the fourth patient was negative by CIE and COA and positive by LA. Among the six patients presenting with a negative culture, two gave a false-negative result by CIE.

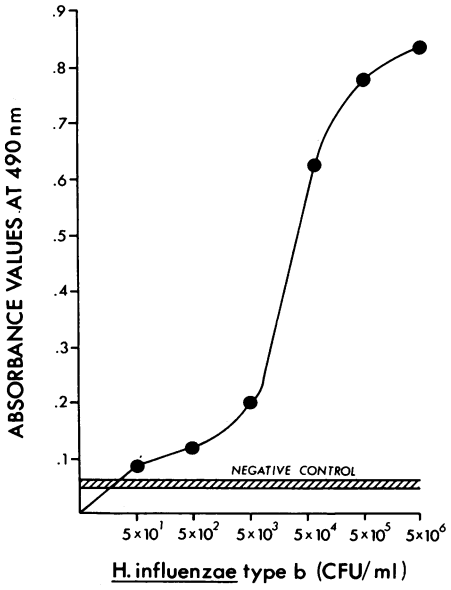

FIG. 2. Titration of *H. influenzae* type b by the B-A EIA test. Each point represents the mean value of three replicates ± standard deviation.

TABLE 1. B-A EIA reactivity of the MAb Hb_1 against a panel of bacterial strains

Microorganism[a] or control	No. of strains tested	A_{490} (mean ± SD)[b]
PBS-Tween 20 (negative control)		0.040 ± 0.003
S. aureus	8	0.055 ± 0.019
Staphylococcus epidermidis	9	0.050 ± 0.018
S. pneumoniae	12	0.061 ± 0.015
S. pyogenes	5	0.050 ± 0.018
Group B *Streptococcus* sp.	17	0.056 ± 0.018
N. meningitidis	11	0.060 ± 0.015
E. coli	9	0.050 ± 0.016
H. influenzae type a	5	0.055 ± 0.011
H. influenzae type d	5	0.060 ± 0.016
H. influenzae type e	5	0.050 ± 0.010
H. parainfluenzae	10	0.054 ± 0.019
H. influenzae type b	20	0.790 ± 0.117

[a] The bacterial strains were isolated from children and used at a concentration of 10^5 CFU/ml.
[b] Mean of triplicates ± standard deviation.

TABLE 2. Comparative study of the detection of *H. influenzae* serotype b antigens in body fluids of infants during severe infection

Body fluid (no. of specimens tested)	No. of specimens positive by the following method (sensitivity[a] [%]):				
	Culture	CIE	COA-Phadebact	LA-Bactigen	B-A EIA
CSF[b] (18)	12	13 (78)	15 (86)	15 (86)	18 (100)
Urine[c] (12)	NT[d]	8 (75)	10 (86)	11 (92)	12 (100)
Serum[e]					
Acute (8)	NT	5 (73)	NT	6 (86)	8 (100)
Convalescent (8)	NT	0	NT	1	5

[a] Sensitivity: number of true-positive specimens/(number of true-positive specimens + number of false-negative specimens) × 100.
[b] CSF specimens were collected from 18 infants presenting with meningitis.
[c] Urine specimens were collected from 12 infants presenting with meningitis and had positive CSF culture results for *H. influenzae* type b.
[d] NT, Not tested or culture not performed.
[e] Serum specimens were collected from eight infants presenting with meningitis (four cases), epiglottitis (two cases), and cellulitis (two cases). All had positive CSF or blood cultures positive for *H. influenzae* type b, except one specimen which did not grow out *H. influenzae* type b on culture but was positive for *H. influenzae* type b antigens by the CIE, LA-Bactigen, and B-A EIA methods.

The specificity of these four serological methods was evaluated by testing 26 CSF specimens collected from 26 pediatric patients presenting with a meningitis caused by a variety of bacteria other than *H. influenzae* type b. No false-positive reaction was observed, except on three occasions when CIE was positive (with *S. pneumoniae*, *E. coli*, and group B *Streptococcus* sp.) and on two occasions when LA-Bactigen was positive (with *S. pneumoniae*), even after heating at 100°C for 3 min. The B-A EIA and the COA-Phadebact test were the only two methods giving no false-positive result.

Sera. We also tested serum collected from eight patients, aged 1 month to 5 years, presenting with a severe infection caused by *H. influenzae* serotype b. Four of these children had meningitis which was documented by positive CSF culture in three and characteristic Gram staining in one. Two children had epiglottitis, and two had cellulitis (all four cases documented by positive blood cultures). A first serum sample was collected after 1 to 4 days of hospitalization. All of these specimens were positive by B-A EIA, six were positive by the LA-Bactigen test, and five were positive by CIE (Table 2). The differences in sensitivity between B-A EIA and CIE were significant, with a P value of 0.083, but were not significant between B-A EIA and LA ($P = 0.157$).

To evaluate the persistence of the antigen in the circulatory system, serum was collected from the same eight patients after a posttherapy period ranging from 3 to 4 weeks and was tested concomitantly with the serum collected during the acute phase. Five samples remained positive by B-A EIA, whereas only one was positive by LA and none were positive by CIE (Table 2). Thus, the B-A EIA was significantly more sensitive than CIE ($P = 0.025$) and LA ($P = 0.046$).

As a negative control, we first tested the serum collected from 14 patients during the acute phase of meningitis caused by *S. pneumoniae* (four cases), *N. meningitidis* (three cases), group B *Streptococcus* sp. (three cases), and *E. coli* (four cases). All of these samples were negative by B-A EIA, whereas one was positive by LA-Bactigen (*S. pneumoniae*) and two were positive by CIE (*S. pneumoniae* and *E. coli*). An additional 45 serum samples collected from healthy children were evaluated by the B-A EIA and found to be negative. These latter serum samples also permitted determination of the negative control of the assay.

Urine. A total of 12 urine samples were collected from 12 children presenting with meningitis caused by *H. influenzae* serotype b during the acute phase and were tested by the four serological methods described. All of these specimens were positive by B-A EIA, whereas 11, 10, and 8 were positive by LA, COA, and CIE, respectively (Table 2). Thus, the B-A EIA was more sensitive than CIE ($P = 0.046$) but not significantly more sensitive than COA ($P = 0.157$) and LA ($P = 0.317$).

As a negative control, we studied urine specimens collected from healthy children (10 cases) and from patients (10 cases) presenting with a variety of infectious diseases caused by microorganisms other than *H. influenzae* type b. The B-A EIA test yielded negative results for all of these specimens, whereas CIE yielded positive results for two specimens and LA-Bactigen and COA-Phadebact yielded positive results for one specimen.

DISCUSSION

In a first set of experiments, we detected *H. influenzae* type b antigens by using both a polyclonal antibody produced in rabbits which had been immunized with whole cells of an *H. influenzae* type b strain as the solid-phase reagent and a commercial peroxidase-labeled anti-mouse immunoglobulin reacting with the *H. influenzae* type b-specific MAb (Hb_1). The major disadvantages of this method were poor sensitivity and reproducibility in addition to a high background activity; i.e., high absorbance values were obtained for normal serum. As a second step, we found that the use of a polyclonal antibody reacting to the PRP as the solid-phase reagent, as well as a peroxidase-labeled MAb (Hb_1) directed against OMP as the liquid-phase reagent, gave better results. Because the solid- and liquid-phase antibodies are directed at different determinants, this system offers the potential advantage that the binding of antigen to the solid phase will not result in a decrease in the number of antigenic sites available to the liquid-phase antibody (31). In a final step, we found that the use of a biotinylated MAb (Hb_1) as the liquid-phase reagent reacting with the peroxidase-linked avidin still increased the sensitivity of this new assay. This last finding is consistent with other reports describing the advantages of the avidin-biotin immunoassay systems (16, 32).

The B-A EIA described in this report could detect as few as 50 *H. influenzae* serotype b organisms per ml grown in broth culture. When applied to clinical specimens (CSF, serum, and urine), this new B-A EIA showed greater sensitivity than did CIE, LA, and COA (Table 2).

In children presenting with meningitis caused by *H. influenzae* serotype b and group B *Streptococcus* sp., the concentrated urine remained antigen positive by LA (*H. influenzae* serotype b) after therapy for 20 days and by CIE (group B *Streptococcus* sp.) after therapy for 21 days (1, 22). In the present study, five serum samples from convalescent patients collected 3 weeks after the acute period remained positive by B-A EIA, whereas only one was positive by LA and none were positive by CIE. This significant difference in the detection of the antigens in the sera of convalescent children with either meningitis or another type of infection is in accordance with a greater sensitivity of this new EIA. The high level of sensitivity of this new test will be very helpful for the management of children presenting with infection at a hospital after receiving antibiotics.

In addition to this high sensitivity, the B-A EIA showed an excellent specificity. In fact, no positive reactions were noted in CSF, serum, or urine specimens collected from children infected with bacteria other than *H. influenzae* type b or from children without evidence of bacterial infection, whereas on three occasions a false-positive reaction was the result of the LA test in three cases of infection with *S. pneumoniae*. Recently, Macone et al. (19) reported that five patients infected with a pathogen other than *H. influenzae* serotype b had a positive Bactigen-LA test. These five cases were represented by *S. pneumoniae* type 14 (two cases), *N. meningitidis* group C (one case), *E. coli* K-100 (one case), and *Staphylococcus aureus* (one case). In our laboratory, we observed five false-positive reactions involving *S. pneumoniae* (two cases), *E. coli* (two cases), and group *B Streptococcus* sp. (one case) by using the CIE method. These false-positive reactions have also been observed after use of an antiserum reacting to PRP (*H. influenzae* type b), to *E. coli* (Easter strain K-100), and to *S. pneumoniae* (type 29) (12). When the CSF specimens were tested by COA, no false-positive results were noted. The bloody CSF specimens or those specimens presenting with a high protein content also reacted nonspecifically with several antigen reagents. On a few occasions, false-positive reactions were

mediated by the presence of anti-staphylococcus antibody in the serum. Premixing with soluble protein A eliminated most of these nonspecific reactions in such cases (27). With the commercial kit recommendations, one can find that heating the specimen at 80°C for 5 min is required to eliminate these inconclusive reactions. It was also reported that the use of polyclonal antibodies in the EIA system for the detection of *H. influenzae* type b antigens could yield a false-positive result (19, 25). Such nonspecific reactions and methods to minimize them have been reviewed by Yolken (31). On the contrary, the MAb used in this new B-A EIA test ensured a highly specific reactivity, as demonstrated by the complete absence of false-positive results. This advent of serotype-specific MAb to *H. influenzae* type b and its use in the EIA system provide a new, powerful technique capable of detecting *H. influenzae* type b antigens in clinical specimens.

ACKNOWLEDGMENTS

We thank P. Tijssen of the Institut Armand Frappier, Laval, Quebec, Canada for his critical review of the manuscript and Roch Roy of the Département d'Informatique et Recherche Opérationnelle, Université de Montréal, for his assistance in statistical analysis.

This work was supported by the Fondation Justine Lacoste-Beaubien and by CAFIR (Université de Montréal). A research studentship was obtained from the Foundation du Conseil des Clubs Sociaux de Montréal (A.B.) and from the Fonds de la Recherche en Santé du Québec (J.H.).

LITERATURE CITED

1. **Baker, C. J., B. J. Webb, C. V. Jackson, and M. S. Edwards.** 1980. Countercurrent immunoelectrophoresis in the evaluation of infants with group B streptococcal disease. Pediatrics **65**:1110–1114.
2. **Bayer, E. A., and M. Wilchek.** 1974. Avidin-biotin interactions. Methods Enzymol. **34**:265–267.
3. **Bradshaw, M. W., R. Schneerson, J. C. Parke, Jr., and J. B. Robbins.** 1971. Bacterial antigens cross-reactive with the capsular polysaccharide of *Haemophilus influenzae* type b. Lancet **i**:1095–1096.
4. **Brodeur, B. R., F. E. Asthon, and B. B. Diena.** 1982. Enzyme-linked immunosorbent assay with polyvalent gonococcal antigen. J. Med. Microbiol. **15**:1–9.
5. **Brodeur, B. R., Y. Larose, P. Tsang, J. Hamel, F. Ashton, and A. Ryan.** 1985. Protection against infection with *Neisseria meningitidis* group B serotype 2b by passive immunization with serotype-specific monoclonal antibody. Infect. Immun. **50**: 510–516.
6. **Brodeur, B. R., P. Tsang, and Y. Larose.** 1984. Parameters affecting ascites tumour formation in mice and monoclonal antibody production. J. Immunol. Methods **71**:265–272.
7. **Colding, H., and I. Lind.** 1977. Counterimmunoelectrophoresis in the diagnosis of bacterial meningitis. J. Clin. Microbiol. **5**:405–409.
8. **Crosson, F. J., Jr., J. A. Winkelstein, and E. R. Moxon.** 1978. Enzyme-linked immunosorbent assay for detection and quantitation of capsular antigen of *Haemophilus influenzae* type b. Infect. Immun. **22**:617–619.
9. **Daum, R. S., G. R. Siber, J. S. Kamon, and R. R. Russel.** 1982. Evaluation of a commercial latex agglutination test for rapid diagnosis of *Haemophilus influenzae* type b infection. Pediatrics **69**:466–471.
10. **Dirks-Go, S. I. S., and H. C. Zanen.** 1978. Latex agglutination, counterimmunoelectrophoresis, and protein A coagglutination in diagnosis of bacterial meningitis. J. Clin. Pathol. **31**: 1167–1171.
11. **Drow, D. L., D. G. Maki, and D. D. Manning.** 1979. Indirect sandwich enzyme-linked immunosorbent assay for rapid detection of *Haemophilus influenzae* type b infection. J. Clin. Microbiol. **10**:442–450.
12. **Feldman, W. E.** 1977. Relation of concentrations of bacteria and bacterial antigen in cerebrospinal fluid to prognosis in patients with bacterial meningitis. N. Engl. J. Med. **296**:433–435.
13. **Finch, C. A., and H. W. Wilkinson.** 1979. Practical considerations in using counterimmunoelectrophoresis to identify the principal causative agents of bacterial meningitis. J. Clin. Microbiol. **10**:519–524.
14. **Fung, J. C., and D. K. Wicher.** 1982. Comparison of slide agglutination test (Phadebact) with counterimmunoelectrophoresis for detection of streptococcal group antigens. J. Clin. Pathol. **77**:608–610.
15. **Harding, S. A., W. M. Scheld, M. D. McGowan, and M. A. Sande.** 1979. Enzyme-linked immunosorbent assay for detection of *Streptococcus pneumoniae* antigen. J. Clin. Microbiol. **10**:339–342.
16. **Kendall, C., I. Ionescu-Matiu, and G. R. Dreesman.** 1983. Utilization of the biotin/avidin system to amplify the sensitivity of the enzyme-linked immunosorbent assay (ELISA). J. Immunol. Methods **56**:329–339.
17. **Köhler, G., and C. Milstein.** 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) **256**:495–497.
18. **Lampe, R. M., T. Chottipitayasunondh, and P. Sunakorn.** 1976. Detection of bacterial antigen in pleural fluid by counterimmunoelectrophoresis. J. Pediatr. **88**:557–560.
19. **Macone, A. B., G. Arakere, J. M. Letourneau, and D. A. Goldmann.** 1985. Comparison of a new, rapid enzyme-linked immunosorbent assay with latex particle agglutination for the detection of *Haemophilus influenzae* type b infections. J. Clin. Microbiol. **21**:711–714.
20. **Montplaisir, S., and J. Bahous.** 1977. Diagnostic rapide des infections bactériennes par la contre-immunoélectrophorèse. Union Méd. Can. **106**:1402–1408.
21. **Newman, R. B., R. W. Stevens, and A. H. Goafar.** 1970. Latex agglutination test for the diagnosis of *Haemophilus influenzae* meningitis. J. Lab. Clin. Med. **76**:107–113.
22. **Riera, L.** 1985. Detection of *Haemophilus influenzae* type b antigenuria by Bactigen and Phadebact kits. J. Clin. Microbiol. **21**:638–640.
23. **Schneerson, R., M. Bradshaw, J. K. Whisnant, R. L. Myerowitz, J. C. Parke, Jr., and J. B. Robbins.** 1972. An *Escherichia coli* antigen cross-reactive with the capsular polysaccharide of *Haemophilus influenzae* type b: occurrence among known serotypes, and immunochemical and biologic properties of *E. coli* antisera toward *H. influenzae* type b. J. Immunol. **108**:1551–1562.
24. **Shaw, E. D., R. J. Darker, W. E. Feldman, B. M. Gray, L. L. Pifer, and G. B. Scott.** 1982. Clinical studies of a new latex particle agglutination test for detection of *Haemophilus influenzae* type b polyribose phosphate antigen in serum, cerebrospinal fluid, and urine. J. Clin. Microbiol. **15**:1153–1156.
25. **Sippel, J. E., C. M. Prato, N. I. Girgis, and E. A. Edwards.** 1984. Detection of *Neisseria meningitidis* group A, *Haemophilus influenzae* type b, and *Streptococcus pneumoniae* antigens in cerebrospinal fluid specimens by antigen capture enzyme-linked immunosorbent assays. J. Clin. Microbiol. **20**:259–265.
26. **Suksanong, M., and A. S. Dajani.** 1977. Detection of *Haemophilus influenzae* type b antigens in body fluids, using specific antibody-coated staphylococci. J. Clin. Microbiol. **5**:81–85.
27. **Thirumoorthi, M. C., and A. S. Dajani.** 1979. Comparison of staphylococcal coagglutination, latex agglutination, and counterimmunoelectrophoresis for bacterial antigen detection. J. Clin. Microbiol. **9**:28–32.
28. **Ward, J. I., G. R. Siber, D. W. Scheifele, and D. H. Smith.** 1978. Rapid diagnosis of *Haemophilus influenzae* type b infections by latex particle agglutination and counterimmunoelectrophoresis. J. Pediatr. **93**:37–42.
29. **Webb, B. J., M. S. Edwards, and C. J. Baker.** 1980. Comparison of slide coagglutination test and countercurrent immunoelectrophoresis for detection of group B streptococcal antigen in cerebrospinal fluid from infants with meningitis. J. Clin. Microbiol. **11**:263–265.

30. **Welch, D. F., and D. Hensel.** 1982. Evaluation of Bactogen and Phadebact for detection of *Haemophilus influenzae* type b antigen in cerebrospinal fluid. J. Clin. Microbiol. **16**:905–908.
30a. **Winer, B. J.** 1971. Statistical principles in experimental design, p. 303. McGraw-Hill Book Co., New York.
31. **Yolken, R. H.** 1982. Enzyme immunoassays for the detection of infectious antigens in body fluids: current limitations and future prospects. Rev. Infect. Dis. **4**:35–68.
32. **Yolken, R. H., F. J. Leister, L. S. Whitcomb, and M. Santosham.** 1983. Enzyme immunoassays for the detection of bacterial antigens utilizing biotin-labeled antibody and peroxidase biotin-avidin complex. J. Immunol. Methods **56**:319–327.