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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

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A new latex particle agglutination test for direct detection of *Haemophilus influenzae* type b polyribose phosphate antigen in serum, cerebrospinal fluid, or urine was evaluated from studies at four clinical centers. Although use of a serum buffer significantly reduced inconclusive agglutination of the latex particles, the retesting of serum samples, after heat inactivation and dilution, resolved all serum samples, with one exception, as reactive or nonreactive for the presence of the polyribose phosphate antigen. A clinical accuracy of 100% was obtained for the latex particle agglutination method in respect to its capability for detection of polyribose phosphate antigen in all patients with confirmed infection by *H. influenzae* type b.

The coagglutination procedure, as designed for identification of *Haemophilus influenzae* cultured from clinical specimens, requires approximately 10^8 bacteria per ml for positive agglutination, but it is superior to the conventional slide agglutination test that requires approximately 10^{10} bacteria per ml (5). Conventional culture techniques require 18 to 24 h with approximately 10^3 colony-forming units per ml in the inoculum. The method of counterimmunoelectrophoresis (CIE) may provide results within 1 h, but its requirement of 10^3 bacterial colony-forming units per ml, or a minimum of 5.0 to 50.0 ng of *H. influenzae* b polyribose phosphate (PRP) antigen per ml of clinical sample does not yield sufficient sensitivity for detection of small amounts of PRP antigen from infections (7, 9).

Concentrations of *H. influenzae* type b bacteria and the PRP antigen in cerebrospinal fluid (CSF) are associated with prognosis in patients with bacterial meningitis. Those patients with meningitis who have relatively large quantities of bacterial antigen in CSF tend to present neurological sequelae, subdural effusions, prolonged fever, and higher morbidity and mortality than in patients with less antigen (3). Also, prior antibiotic therapy significantly decreases the quantity of *H. influenzae* type b in CSF, which diminishes the usefulness of the Gram stain with decreased yield of positive cultures (4). With the latex particle agglutination (LPA) technique, the PRP antigen can be detected in very small amounts in specimens containing 10^2 or less bacteria per ml when taken during the early stages of infection and as excreted during early convalescence after the culture samples have become negative (7).

Presented here are results of clinical studies using an antibody-coated LPA test. This test system is commercially available (Wampole Laboratories, Cranbury, N.J.) as a 10-min slide LPA test at room temperature. It has provided a very sensitive technique for qualitative and quantitative determination of the *H. influenzae* type b bacterial antigen (from 0.2 to 1.0 ng/ml) in specimens of serum, CSF, or urine taken from infected patients.

The studies included performance of the LPA test on specimens from patients at four major clinical centers. In each case of *H. influenzae* type b bacterial meningitis, or infections such as sepsis, epiglottitis, pneumonia alone or combined with meningitis, or bacteremia, the diagnosis was confirmed by either culture or CIE methods.

Reagents for the LPA test consisted of the following: (i) latex particles were sensitized with equine (anti-*H. influenzae* type b) serum globulin, and after they were washed with a stabilizing buffer, the particles were suspended into a pro-

TABLE 1. Comparison of three methods used in detection of *H. influenzae* type b in specimens from confirmed cases of infection

Clinical study (no. of specimens)	Culture method					CIE method					LPA method				
	n^a	+[b]	−[c]	NT[d]	Clinical accuracy[e]	n	+	−	NT	Clinical accuracy	n	+	−	NT	Clinical accuracy
64 Cases															
CSF (80)	78	42	36	2	54% (42/78)	19	6	13	61	32% (6/19)	80	80	0	0	100% (80/80)
Serum (35)	31	22	9	4	71% (22/31)	8	5	3	24	63% (5/8)	35	35	0	0	100% (35/35)
Urine (38)	14	4	10	24	29% (4/14)	2	2	0	36	NA[f]	38	38	0	0	100% (38/38)
Follow-up (asymptomatic)															
CSF (2)	2	0	2	0	NA	0	0	0	2	NA	2	2	0	0	NA
Serum (16)	13	1	12	3	NA	3	0	3	13	NA	16	16	0	0	100% (16/16)
Urine (15)	12	0	12	3	NA	0	0	0	15	NA	15	15	0	0	100% (15/15)

[a] n, Number of specimens examined.
[b] +, Positive or reactive.
[c] −, Negative or nonreactive.
[d] NT, Not tested.
[e] Clinical accuracy with respect to indicated method used for detection of PRP antigen or bacteria in specimens from patients with confirmed infection by *H. influenzae* type b.
[f] NA, Not applicable.

tein-stabilized buffer; (ii) control latex particles were sensitized with nonimmune equine serum globulin, washed, and suspended as above; and (iii) an *H. influenzae* type b PRP standard was purified (1), and a negative PRP control was included along with a serum buffer, which minimized nonspecific agglutination by serum antiglobulins and inhibited binding by human complement components (9, 10). The freeze-dried preparations of *H. influenzae* type b PRP antigen (standardized at 2.0 ng PRP per ml) and of serum buffer were reconstituted with a protein-stabilized diluent at pH 8.2.

Assays were performed on clear glass serological ring slides. The clinical sample was added in 50-μl volumes to duplicate wells with a 10-μl portion of antibody-coated latex particles mixed into one well and a 10-μl portion of control latex particles mixed into the second well. For serum specimens, but not for CSF or urine, 10 μl of reconstituted serum buffer was added to the serum wells before mixing with the latex preparations. A negative PRP control and reconstituted PRP standard were included with each set of test samples. After rotation of 140 rpm on a serological rotary apparatus at room temperature for ten minutes, the slides were removed and examined over oblique light above a black background for the agglutination patterns. Infrequently, a serum specimen yielded an inconclusive agglutination of antibody-coated and control latex particles. If this occurred, the serum specimen was retested after heat inactivation at 56°C for 30 min and dilution at 1:5 in the diluent. All agglutination reactions were resolved as either specifically reactive or nonreactive. In one study, positive CSF specimens were incubated in a boiling water bath for 2 min to resolve nonspecific agglutination, which occurred infrequently with CSF specimens. Quantitative assays for PRP antigen were performed by dispensing 50-μl volumes of clinical samples, and PRP antigen was serially diluted in parallel for reaction with the antibody-coated latex particles in the 10-min test. The concentration, expressed in nanograms of PRP antigen per milliliter, was determined by multiplication of the endpoint titer (reciprocal of the last dilution) of test specimen which reacted at a +1 or +2 agglutination by the defined nanograms of PRP antigen per milliliter at the endpoint reading for the diluted PRP standard.

From our clinical studies of 82 CSF, 51 sera, and 53 urine samples from 64 cases of infection with *H. influenzae* type b, as confirmed by either culture or CIE techniques, bacterial cultures were positive in 51 and negative in 13 cases. Although the CIE technique was not routinely performed at all four clinical centers, the 13 cases that were negative by culture were

confirmed with the CIE technique. In 937 specimens from 889 patients with a wide variety of other infections, plus serum specimens from 180 healthy individuals, both the LPA assay and cultures were negative for *H. influenzae* type b. Other types of infection included 18 cases of viral or aseptic meningitis, 12 cases of meningitis of unknown etiology, 4 of meningococcemia, 6 cases of *Streptococcus pneumoniae* (serotype 6), 6 cases of meningococcal meningitis, 5 cases of group B streptococcal meningitis, and 1 case each of meningoencephalitis, *Escherichia coli* meningitis, and *Salmonella* meningitis. The LPA assays and all cultures were negative for *H. influenzae* type b in these 889 cases with other types of infection.

As indicated in Table 1, a 100% clinical accuracy with respect to capability of a method to detect the bacteria or antigen during infection of a patient was established for the LPA reagents for direct detection of *H. influenzae* type b PRP antigen in all three types of 186 specimens from 64 cases confirmed by either culture or CIE methods. Although many laboratories have low yields with conventional culture techniques, only those cases of infection, as confirmed by either culture or CIE were included for evaluation. Values for clinical accuracy were obtained for expected culture results. From 80 CSF specimens, only 42 of 78 cultured specimens were positive for *H. influenzae* type b which yielded a clinical accuracy of 54% for the culture detection technique. There also were values of 71% (22 of 31) and 29% (4 of 14) for clinical accuracy, respectively, with serum and urine cultures. Thus, if only the culture method is used, viable bacteria at approximate concentrations of 10^3 or more in a clinical sample will be detected at the estimated levels of accuracy. By using similar criteria for the CIE technique, which also requires approximately 10^3 colony-forming units of bacteria per ml for the detection of PRP antigen, the values of 32 per 100 specimens of CSF and of 63 per 100 specimens of serum can be applied. Results from examination of specimens taken during convalescence for patients who became asymptomatic but were positive for *H. influenzae* type b in earlier specimens reflect the diminution of viable bacteria for culture and of PRP antigen for detection with the less sensitive CIE method.

Although the serum buffer significantly reduced the inconclusive or nonspecific agglutination of the control latex (nonimmune globulin-coated latex particles) by serum samples, approximately one of every six (16%) serum specimens required retesting for the removal of inconclusive agglutination. From our data, 12% (5 of 42) of the serum specimens were resolved as specifically reactive for *H. influenzae* type b PRP antigen by retesting. In one study, however, one inconclusive result (i.e., the agglutination of both antibody-coated and control latex particles) was obtained in a serum sample from a patient having pneumonia, otitis media, and conjunctivitis with *H. influenzae* type b previously cultured from the eye infection. No false-positive and no false-negative LPA tests occurred in these four controlled clinical evaluations.

A case history of a 4-month-old infant with diagnosis of meningitis was selected from the studies of author L.L.P. to illustrate an application for the quantitative LPA assay. Initial therapy included ampicillin and chloramphenicol with ampicillin discontinued on day 4. The concentration of *H. influenzae* type b PRP antigen was determined in several clinical specimens of CSF, serum, or urine collected over the 14 days from the initial point of entry into the study. The PRP antigen decreased from 3,125 ng/ml in the initial serum (day 2 of the study) to 25 ng/ml 9 days later. The PRP antigen increased fivefold, however, from 25 ng/ml in urine (day 5) to 125 ng/ml 3 days later, with negative cultures for *H. influenzae* type b not defining a primary urinary tract infection. LPA tests and cultures for *H. influenzae* type b were positive in three samples of CSF collected on day 1 and in one serum taken on day 2. The cultures were negative from samples of urine and serum on days 5, 8, and 11, but they were reactive by LPA for the PRP antigen. One CSF sample collected on day 14 of study was negative for culture and nonreactive for PRP antigen by the LPA technique.

Cross-reactivity of rabbit, goat, or sheep antibody for *H. influenzae* type b has been described using serum-in-agar techniques with several serotypes of *S. pneumoniae* (2, 6, 8). From laboratory studies of in vitro stock culture suspensions, *S. pneumoniae* serotype 29 and *E. coli* (Easter strain) were reactive with the LPA reagents. No agglutination, however, was observed by the LPA reagents with pneumococcal polysaccharide antigens of serotypes 1, 3, 4, 6A, 7A, 8, 9N, 12, 14, 18, or 19 at concentrations of 1.0 μg/ml.

On the basis of four prospective clinical evaluations, the LPA technique can be accepted as an excellent adjunctive and specific test in early diagnosis of bacterial meningitis due to *Haemophilus influenzae* type b. The sensitivity of the LPA technique presents a distinct advantage over conventional methods of culture or CIE for early diagnosis of this disease.

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