JOURNAL OF CLINICAL MICROBIOLOGY, June 1987, p. 1131–1133
0095-1137/87/061131-03$02.00/0

Vol. 25, No. 6

Latex Agglutination Test for Screening of *Haemophilus influenzae* Type b Carriers

RITVA HEIKKILÄ, AINO TAKALA, HELENA KÄYHTY, AND MAIJA LEINONEN*

National Public Health Institute, SF-00280 Helsinki, Finland

Received 10 December 1986/Accepted 16 February 1987

Latex agglutination (LA) for the demonstration of *Haemophilus influenzae* type b capsular antigen in nasopharyngeal swabs was applied to the screening of *H. influenzae* type b carriers and compared with culturing by the antiserum agar method on Levinthal-bacitracin plates. Altogether, 51 (33%) of the 154 nasopharyngeal specimens collected from the close contacts of patients with invasive *H. influenzae* type b disease were positive in LA or the antiserum agar method or both. All 40 culture-positive samples were also positive in LA, and in 11 (9.6%) culture-negative samples, *H. influenzae* type b antigen was demonstrated by LA. LA as used in the present study is cheap, rapid, and easy to perform and is thus an ideal tool for the screening of *H. influenzae* type b carriers.

Preschool-aged family contacts of patients with *Haemophilus influenzae* type b infections have an increased risk for secondary *H. influenzae* type b infections (2, 5, 19; J. Eskola, A. Takala, H. Käyhty, M. Leinonen, T. Kilpi, H. Peltola, and P. H. Mäkelä, J. Infect., in press). The adherence of *H. influenzae* type b to the nasopharyngeal epithelium, followed by colonization and invasion of the organism, is thought to be the preliminary step in the pathogenesis of invasive disease (1). Rapid screening of nasopharyngeal carriers could thus detect contacts at risk. Screening of *H. influenzae* type b carriers is also needed for epidemiological surveys. However, the use of nasopharyngeal culture methods for screening is often problematic. Firstly, routine culture methods are not suitable for screening of *H. influenzae* type b, whereas the recommended method, culturing with antiserum agar (ASA) on Levinthal-bacitracin plates, is available in a few specialized laboratories only (13). Secondly, the ASA method requires large quantities of *H. influenzae* type b antiserum and is thus a very expensive method. Thirdly, culturing and identifying of *H. influenzae* type b strains takes at least 2 days.

Rapid antigen detection methods, such as the latex agglutination (LA) method, have been widely used for the diagnosis of septicemia, meningitis, and pneumonia caused by *H. influenzae* type b, meningococci, or pneumococci (3, 8, 9, 11), and these methods have been shown to be very specific and sensitive. Recently, LA tests have also been applied to the detection of streptococcal group A antigen (and, indirectly, group A streptococci) from throat swabs of patients with tonsillitis (4, 20).

The aim of the present study was to test the application of the LA test to the demonstration of *H. influenzae* type b antigen in nasopharyngeal swabs as a screening method for *H. influenzae* type b carriers. This method was compared with culturing by the ASA method on Levinthal-bacitracin plates.

We collected nasopharyngeal specimens from close contacts of 47 patients with invasive *H. influenzae* type b disease in different parts of Finland from June 1985 to April 1986. Altogether, 154 specimens were obtained from family members of these patients. After identification of an index case with *H. influenzae* type b disease, pediatricians informed parents about the study and asked their consent to a home visit. One of the two project nurses visited the family as soon as possible, usually within 2 to 3 days, and collected specimens from the nasopharynges of family members with charcoal-coated swabs, which were immersed immediately into Stuart transport medium (Transpocult; Orion Diagnostica, Espoo, Finland).

The specimens were spread within 12 h onto Levinthal-bacitracin agar plates (13) containing 2.75 ml of *H. influenzae* type b antiserum (burro 46; kindly provided by John B. Robbins, National Institutes of Health, Bethesda, Md.) in 45 ml of agar. The ASA plates were stored before use at 4°C in tightly closed plastic bags for not more than 2 weeks. The plates were incubated after inoculation at 35°C in an atmosphere containing 10% CO_2. An *H. influenzae* type b control strain as well as a strain of nontypable *H. influenzae* were included every time patient samples were tested. The plates were inspected for precipitation haloes around colonies after 24 and 48 h of incubation. The colonies with precipitation haloes were confirmed to be *H. influenzae* type b by LA (Wellcogen *H. influenzae* type b; Wellcome Diagnostics, Dartford, England).

After being cultured, the swabs were returned to the Transpocult tubes and stored at −20°C. For the LA tests, the swabs were taken from the thawed transport medium, placed in 0.5 ml of phosphate-buffered saline, pH 7.4, mixed vigorously with a Vortex mixer, and centrifuged (2,000 × *g*) for 5 min. The supernatants were then heated in a boiling water bath for 5 min. The LA tests were performed with the supernatants in accordance with the instructions of the manufacturer (Wellcome) by using both test and control latex reagents. In some cases, the liquid formed in the Transpocult tubes after freezing was boiled and tested for *H. influenzae* type b antigen by LA.

Table 1 shows the comparison of the LA test with ASA culturing. The presence of *H. influenzae* type b in nasopharyngeal samples could be demonstrated by culturing in 40 (26%) of the 154 specimens tested. All culture-positive samples were also positive for *H. influenzae* type b antigen

* Corresponding author.

TABLE 1. Comparison of LA and ASA culturing for demonstration of *H. influenzae* type b in throat samples

LA result	Culture result (no.)		
	Positive	Negative	Total
Positive	40	11	51
Negative	0	103	103
Total	40	114	

in the LA test, so the sensitivity of the LA test in comparison with culturing was 100%. Eleven (9.6%) of the culture-negative samples were positive for *H. influenzae* type b antigen in the LA test. Thus, the presence of *H. influenzae* type b could be demonstrated in 51 (33%) of the throat samples tested. The 51 *H. influenzae* type b cases came from 29 (61%) of the 47 families assayed. In most cases (21 families), one or more siblings were *H. influenzae* type b carriers. In eight families, only adult carriers were demonstrated; the carrier was a mother in five cases and a father in three cases.

The LA test was shown to be highly sensitive (100%) for the demonstration of *H. influenzae* type b strains in nasopharyngeal specimens. In addition, there were 11 cases (9.6%) that were LA positive but culture negative. It is of course possible that these were false-positive findings. Bacteria with polysaccharides that are cross-reactive with *H. influenzae* type b may be present in throat samples: some pneumococci and *Escherichia coli* K100 have capsules that closely resemble the *H. influenzae* type b capsule (14, 15). The presence of these kinds of bacteria in throat samples was not carefully excluded by the culture methods used in the present study, but in general such strains are uncommon. However, it is more probable that the *H. influenzae* type b antigen is present in nasopharyngeal samples in which the bacteria have lost viability through, e.g., antibiotic treatment prior to sampling, prolonged transportation of the sample, or the action of antibodies or phagocytes. In a possibly analogous situation, we have shown that pneumococcal antigens are detected in 20 to 30% of culture-negative middle ear fluid samples during otitis media (10). In addition, we have tested most of the LA-positive, culture-negative samples in an enzyme immunoassay for *H. influenzae* type b antigen developed in our laboratory and obtained positive results (unpublished data). Thus, it seems justified to suggest that the 11 LA-positive, culture-negative samples demonstrate the presence of *H. influenzae* type b strains in nasopharyngeal samples. The LA test used in the present study is easy to perform, rapid, and much cheaper than the ASA method. It is thus a useful tool for the screening of *H. influenzae* type b carriers.

The carriage rate of *H. influenzae* type b strains in healthy populations has been shown to be less than 5% (12, 18). However, the carrier rate in family members of patients with *H. influenzae* type b disease is much higher (10 to 30%), and in orphanages, day-care centers, and hospitals with very close contacts among young children, even higher carrier rates, 30 to 70%, have been reported (6, 7, 16, 17). In the present study, we found an *H. influenzae* type b carrier rate of 33% in family contacts; one or more carriers were found in about half of the families of *H. influenzae* type b patients. The epidemiological aspects of *H. influenzae* type b infection are of great interest. The occurrence of secondary cases of invasive *H. influenzae* type b disease among close contacts of an index case has been repeatedly reported (2, 5, 19; Eskola et al., in press).

This work was partially supported by the World Health Organization Programme on Vaccine Development (contract V8/181/4) and by Connaught Laboratories, Swiftwater, Pa.

We thank Kaija Vilen and Anja Ratilainen for technical assistance, Maija Hakulinen and Pirjo-Riitta Rönnberg for taking nasopharyngeal swabs, and Kaija Helisjoki for secretarial assistance.

LITERATURE CITED

1. **Beachey, E. H.** 1981. Bacterial adherence: adhesin receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. **143**:325–345.
2. **Campbell, L. R., A. J. Zedd, and R. H. Michaels.** 1980. Household spread of infection due to *Haemophilus influenzae* type b. Pediatrics **66**:115–117.
3. **Drow, D. L., and D. D. Manning.** 1983. Comparison of coagglutination, latex agglutination and enzyme-linked immunosorbent assay for detection of *Haemophilus influenzae* type b infection. Diagn. Microbiol. Infect. Dis. **1**:317–322.
4. **Edwards, E. A., I. A. Phillips, and W. C. Suiter.** 1982. Diagnosis of group A streptococcal infections directly from throat secretions. J. Clin. Microbiol. **15**:481–483.
5. **Filice, C. A., J. S. Andrews, Jr., M. P. Hudgins, and D. W. Fraser.** 1978. Spread of *Haemophilus influenzae* type b: secondary illness in household contacts of patients with *Haemophilus influenzae* type b meningitis. Am. J. Dis. Child. **132**:757–759.
6. **Good, P. G., M. D. Fousek, M. F. Grossman, and P. L. Boisvert.** 1943. A study of the familial spread of *Haemophilus influenzae* type b. Yale J. Biol. Med. **15**:913–918.
7. **Greenfield, S., G. Peter, V. M. Howie, J. H. Ploussard, and D. H. Smith.** 1972. Acquisition of type-specific antibodies to *Haemophilus influenzae* type b. J. Pediatr. **80**:204–208.
8. **Kaldor, J., R. Asznowixz, and D. G. P. Breist.** 1977. Latex agglutination in diagnosis of bacterial infections, with special reference to patients with meningitis and septicemia. Am. J. Clin. Pathol. **68**:284–289.
9. **Käyhty, H., P. H. Mäkelä, and E. Ruoslahti.** 1977. Radioimmunoassay of capsular polysaccharide antigens of groups A and C meningococci and *Haemophilus influenzae* type b in cerebrospinal fluid. J. Clin. Pathol. **30**:831–833.
10. **Leinonen, M.** 1980. Detection of pneumococcal capsular polysaccharide antigens by latex agglutination, counterimmunoelectrophoresis, and radioimmunoassay in middle ear exudates in acute otitis media. J. Clin. Microbiol. **11**:135–140.
11. **Leinonen, M., and E. Herva.** 1977. The latex agglutination test for the diagnosis of meningococcal and *Haemophilus influenzae* type b meningitis. Scand. J. Infect. Dis. **9**:187–191.
12. **Michaels, R. H., C. S. Poziviak, F. E. Stonebraker, and C. W. Norden.** 1976. Factors affecting pharyngeal *Haemophilus influenzae* type b colonization rates in children. J. Clin. Microbiol. **4**:413–417.
13. **Michaels, R. H., and F. E. Stonebraker.** 1975. Use of antiserum agar for detection of *Haemophilus influenzae* type b in the pharynx. Pediatr. Res. **9**:513–516.
14. **Robbins, J. B., E. C. Gotschlich, T. Y. Liu, R. Schneerson, Z. T. Handzel, M. Argaman, J. C. Parke, and R. L. Myerowitz.** 1974. Bacterial antigens cross-reactive with the capsular polysaccharides of *Haemophilus influenzae* type b, *Neisseria meningitidis* groups A and C and *Diplococcus pneumoniae* types I and III, p. 39–44. *In* J. B. Robbins, R. E. Horton, and R. M. Krause (ed.), New approaches for inducing natural immunity to pyogenic organisms. U.S. Department of Health, Education, and Welfare publication no. (NIH) 74-553. National Institutes of Health, Bethesda, Md.
15. **Schneerson, R. S., and J. B. Robbins.** 1975. Induction of serum *Haemophilus influenzae* type b capsular antibodies in adult volunteers fed cross-reacting *E. coli* O75:K100:H5. N. Engl. J. Med. **292**:1093–1096.
16. **Turk, D. C.** 1963. Naso-pharyngeal carriage of *Haemophilus influenzae* type b. J. Hyg. **61**:247–256.

17. **Turk, D. C.** 1975. An investigation of the family background of acute haemophilus infections of children. J. Hyg. **75**:315–332.
18. **Turk, D. C.** 1982. Clinical importance of *Haemophilus influenzae*—1981, p. 3–9. *In* S. H. Sell and P. F. Wright (ed.), *Haemophilus* influenza: epidemiology, immunology and prevention of disease. Elsevier Science Publishing Co., Inc., New York.
19. **Ward, J. I., D. W. Frazer, L. J. Baraff, and B. D. Plikaytis.** 1979. *Haemophilus influenzae* type b meningitis: a national study of secondary spread in household contacts. N. Engl. J. Med. **301**:122–126.
20. **White, C. B., J. W. Bass, and S. M. Yamada.** 1986. Rapid latex agglutination compared with the throat culture for the detection of group A streptococcal infection. Pediatr. Infect. Dis. **5**:208–212.