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Serological Grouping of Meningococci and Encapsulated *Haemophilus influenzae* Strains by Latex Agglutination

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The latex agglutination method, utilizing antibody-coated latex particles, was adapted for serogrouping of *Neisseria meningitidis* and serotyping of encapsulated *Haemophilus influenzae* strains from agar plates. It was found to give more clear-cut results than conventional slide agglutination. A 100% agreement with the antiserum agar method was found for all strains isolated from blood or cerebrospinal fluid. Many meningococcal strains from nasopharyngeal carriers are autoagglutinable, but some of these gave a positive reaction with the group B latex reagent, although they were negative by the antiserum agar method. The latex agglutination method has several advantages over others: the lack of autoagglutination, easy performance, easy interpretation, and very low consumption of antisera.

The standard technique for serogrouping of *Neisseria meningitidis* and typing of encapsulated *Haemophilus influenzae*, i.e., bacterial agglutination on glass slides, has definite drawbacks. The agglutination may be weak and ambiguous depending on the growth conditions of the bacteria. This problem, coupled with the spontaneous agglutination so frequently seen, makes the method unreliable except in experienced hands, and even then there is uncertainty in interpretation. Both these difficulties are overcome by the antiserum agar method (ASA), in which antiserum incorporated in the growth medium gives a halo of precipitation around the colonies (2, 3, 8, 13-15). However, the ASA requires very large amounts of antiserum.

We have previously used latex agglutination (LX) with antibody-coated latex particles to detect *H. influenzae* and *N. meningitidis* capsular antigens in cerebrospinal fluid (CSF), and we have found it easy both to perform and to interpret (9, 10). We have now applied it to serogrouping or serotyping of bacteria grown on agar plates and found it very useful, as reported here.

MATERIALS AND METHODS

Bacteria. Sixty-four strains of *N. meningitidis* were isolated by several diagnostic laboratories from the CSF or blood of patients and were sent to the Department of Bacteriology and Immunology, University of Helsinki, for serological group determination. In addition, we tested 36 nasopharyngeal meningococci from healthy persons, collected in a screen for meningococcus carriers. The isolation and bacteriological characterization of these strains have been described earlier (15).

H. influenzae strains included a total of 100 isolates. Of these, 25 were isolated from CSF or blood as described above and were encapsulated. The remaining 75 strains were isolated in the Public Health Laboratory, Oulu, from middle ear aspirates of patients with otitis media; most of these were noncapsulated.

The bacterial strains were prepared for slide agglutination and LX by culture on chocolate agar plates (Mueller-Hinton medium) and incubated overnight at 37°C in a 5 to 10% CO_2 atmosphere.

Antisera. Meningococcal antisera were purchased from Burroughs-Wellcome Co., New York, N.Y. (group B) or Difco Laboratories, Detroit, Mich. (groups A, C, X, Y, and Z), or were received from J. B. Robbins, Bureau of Biologics, Food and Drug Administration, Bethesda, Md. (group A: horse 49; group B: horse 46; and group C: burro 211). Antisera to *H. influenzae* types a to f were purchased from Hyland (Division of Travenol Laboratories, Inc., Costa Mesa, Calif.); type b antiserum (burro 132) was also received from J. B. Robbins.

In some cases (as specified in the text) the antisera were absorbed with polysaccharides. For this purpose, 1 volume of antiserum and 1 volume of the polysaccharide solution, containing 2 mg of polysaccharide in 1 ml of 0.9% saline, were mixed and incubated at 37°C for 2 h.

Meningococcal capsular polysaccharides. Purified high-molecular-weight group B polysaccharide was received from R. H. Tiesjema, Rijks Instituut, Bilthoven, the Netherlands, and the tyramine derivative of group C polysaccharide was provided by E. Gotschlich, The Rockefeller University, New York, N.Y. A mixture of polysaccharides from groups A and C (Meningovax-AC) was from Merck Sharp & Dohme, West Point, Pa.

Agglutination. Agglutination was performed on glass slides with commercial antisera. In some cases the slide agglutination was confirmed by H. A. Feld-

man, Upstate Medical Center, Syracuse, N.Y., who also identified one group 29 E and one W 135 strain of meningococci.

ASA method. Meningococcal serogrouping by this method was performed exactly as described previously (15), using the same horse and burro antisera. *H. influenzae* type b strains were serotyped on Levinthal agar plates (1) containing the burro 132 *H. influenzae* type b antiserum.

LX method. Latex particles (Difco) were sensitized with antibodies by incubating equal volumes of antibody solution (diluted 1:40 with sodium glycine buffer, pH 8.2) and latex suspension in a 37°C water bath for 2 h. Then, an equal volume of 0.1% albumin solution in sodium glycine buffer was added. The latex reagents were stored at 4°C. Latex-MenA, -MenB, and -MenC for groups A, B, and C meningococci, respectively, were prepared from the corresponding horse or burro antisera received from J. B. Robbins. *N. meningitidis* group B antiserum contained some anti-group C reactivity and was therefore absorbed with the mixture of group A and C capsular polysaccharides. Latex-Hia through -Hif reagents for *H. influenzae* encapsulated strains of types a through f were prepared from the commercial antisera.

The tests were performed by placing 1 to 2 drops of the latex reagents on a black plastic plate or on a glass slide and mixing with organisms scraped from one bacterial colony. The slides were tilted for 1 to 2 min and examined against a dark background for agglutination. All tests were performed without prior knowledge of the results of the other methods.

Counterimmunoelectrophoresis. Counterimmunoelectrophoresis was done according to Greenwood et al. (5).

RESULTS

When the bacterial growth was mixed with the corresponding latex reagent, agglutination occurred almost instantaneously, whereas the mixture with the other reagents remained milky. The agglutination was more clear-cut and easier to read than the usual reaction in bacterial slide agglutination.

All 25 *H. influenzae* strains isolated from blood or CSF were of type b and gave the same results with all three methods, bacterial agglutination, ASA, and LX (Table 1). Of 75 *H. influenzae* isolated from middle ear exudates, 6 were found to be type b by all three methods. One strain was classified as type a and one as type f both with LX and bacterial agglutination, whereas 67 reacted with none of the reagents. The latex reagents for *H. influenzae* therefore appeared very satisfactory.

Early in this study, we noticed that the Latex-MenB reagent gave positive reaction with all strains positive with Latex-MenC. It also gave a heavy agglutination with purified group C polysaccharide. A reaction with group C polysaccharide could also be seen as a heavy precipitin line in counterimmunoelectrophoresis with the group B horse antiserum used for preparing the Latex-MenB reagent, suggesting that it contained some anti-group C polysaccharide antibodies. When Latex-MenB was prepared using antiserum absorbed with group A+C polysaccharide, the reaction with group C strains was eliminated. All the Latex-MenB used in the present study was prepared from the absorbed antiserum. The anti-group C polysaccharide activity was not detected when the same group B antiserum was used in the ASA plate method for testing group C meningococci, perhaps because of different sensitivities of the two methods.

Table 2 shows the results of LX tests compared with the ASA method and bacterial slide agglutination for grouping meningococcal strains from clinical specimens (CSF or blood). All group A, B, and C strains gave concordant results with all three methods. Four strains of group Y, two strains of group Z, and three that did not agglutinate by slide agglutination gave negative results in both ASA and LX, in which only anti-A, -B, and -C reagents were used. Some discrepancies were seen with nasopharyngeal *N. meningitidis* strains from carriers (Table 3). Group A and B strains (two and six strains, respectively) gave the same results with all three methods. LX and ASA reactions (for groups A, B, and C) were negative with the five strains belonging to groups X, Y, or W 135. One group 29 E strain gave a positive reaction with Latex-MenB. Most of the nasopharyngeal strains were saline agglutinable. All 20 of these were negative on the ASA plates. Eight of them, however, gave

TABLE 1. *Comparison of LX with ASA and bacterial slide agglutination methods in serotyping H. influenzae strains*

No. of strains tested	ASA[a]	No. of strains positive with LX						Bacterial agglutination
		Hia	Hib	Hic	Hid	Hie	Hif	
25[b]	25	0	25	0	0	0	0	All 25: type b
75[c]	75	1	6	0	0	0	1	Concordant with LX

[a] Number of strains positive on anti-type b plates.
[b] Isolated from blood and CSF cultures.
[c] Isolated from middle ear aspirates.

TABLE 2. *Results of bacterial slide agglutination, ASA, and LX in serogrouping of meningococci isolated from CSF or blood of patients*

N. meningitidis group[a]	No. of strains tested	No. of strains positive with:			
		ASA[b]	Latex-MenA[c]	Latex-MenB[c]	Latex-MenC[c]
A	20	20	20	0	0
B	16	16	0	16	0
C	19	19	0	0	19
Y	4	0	0	0	0
Z	2	0	0	0	0
Nongroupable	3	0	0	0	0

[a] Serogrouping based on results of bacterial slide agglutination using commercial antisera for groups A, B, C, Y, and Z.

[b] Using horse 49 anti-A, horse 46 anti-B, or burro 211 anti-C antisera.

[c] Latex reagents for groups A, B, and C prepared from the same sera used in ASA with the exception that the horse 46 anti-B serum was absorbed with group A+C polysaccharide.

TABLE 3. *Serogrouping of nasopharyngeal meningococci from carriers with LX, slide agglutination, and ASA methods*[a]

N. meningitidis group[b]	No. of strains tested	No. of strains positive with:			
		ASA	Latex-MenA	Latex-MenB	Latex-MenC
A	2	2	2	0	0
B	6	6	0	6	0
X	3	0	0	0	0
Y	1	0	0	0	0
29 E[c]	1	0	0	1	0
W 135[c]	1	0	0	0	0
Saline agglutinators[c]	20	0	0	8	0

[a] Same reagents as in Table 2.

[b] Serogrouping based on bacterial slide agglutination.

[c] Groups 29 E and W 135 and eight of the saline agglutinators were also examined by Harry A. Feldman, Upstate Medical Center, Syracuse, N.Y.

a positive reaction with Latex-MenB, whereas 12 were negative with all three latex reagents. The eight exceptional strains were sent to H. A. Feldman for serogroup confirmation. He also found them to be saline agglutinable.

In an effort to find out the origin of these discrepancies, we studied the Latex-MenB positive, ASA-negative, and saline-agglutinable strains, together with clear-cut group B and C meningococci, with latex reagents prepared from group B and group C antisera that were either unabsorbed or absorbed with group B or group A+C polysaccharides (Table 4). It will be noted that absorption with the group A+C polysaccharide eliminated the reaction of both reagents with group C strains but not the reactions of the Latex-MenB with group B or with the saline-agglutinable strains. Both of the latter reactions disappeared, however, after absorption with group B polysaccharide, suggesting that in both cases the reaction was due to the group B capsular polysaccharide.

DISCUSSION

The serological grouping of meningococcal and *H. influenzae* strains isolated from clinical specimens as well as from carriers is important for epidemiological studies and vaccination program planning. The majority of the clinical strains are easily grouped or typed with conventional slide agglutination, whereas the carrier strains may be nongroupable, because they are either agglutinable even in saline solution or nonagglutinable with any of the antisera.

In serotyping *H. influenzae* strains the LX method was always in agreement with the two reference methods, bacterial slide agglutination and ASA. All *H. influenzae* type b strains from CSF or blood gave the same result with the three methods. Also *H. influenzae* strains from middle ear aspirates, where most strains are nontypable and only 5 to 10% have been type b (6, 7, 11), gave clear-cut results: 6 of 75 strains (8%) were found to be type b by all the methods. One strain was classified as type a and one as type f by both LX and bacterial agglutination. It has been reported (7, 17) that noncapsulated *H. influenzae* strains may give agglutination with antisera, and slide agglutination results are difficult to interpret. In LX no spontaneous agglutination with more than one of the reagents was noticed, and results were easy to interpret.

The LX method also gave 100% correlation with ASA and bacterial agglutination methods in serogrouping meningococcal strains isolated

TABLE 4. *LX reactions with reagents prepared from anti-group B or anti-group C meningococcal antisera with or without absorption*

Meningococcal isolates	Horse 46 anti-B absorbed with[a]:			Burro 211 anti-C absorbed with[a]:		
	None	B	A+C	None	B	A+C
Group B	+	−	+	−	−	−
Group C	+	−	−	+	+	−
Irregular[b]	+	−	+	−	−	−

[a] Absorption with the capsular polysaccharide preparation indicated.

[b] The nasopharyngeal meningococcal isolates of Table 3 that were positive with the Latex-MenB reagent and saline agglutinable.

from blood or CSF. The coagglutination method, which utilizes antibody-coated staphylococci instead of antibody-coated latex particles, has also been shown to be concordant with bacterial slide agglutination of clinical isolates of meningococci (4, 12, 18).

Some discrepancies were noticed when testing meningococcal strains from carriers. When compared with bacterial slide agglutination, LX gave more clear-cut results: saline-agglutinable strains were either negative with all three latex reagents or positive with Latex-MenB. Olcén et al. (12) have also obtained more clear-cut results using coagglutination rather than bacterial agglutination with nasopharyngeal meningococci.

There may be several reasons for the discrepancies between LX and ASA methods. The antisera for all these reactions are prepared by immunizing with whole bacteria and thus contain antibodies not only to the group-specific capsular polysaccharides but also to other cell components. In ASA only capsular polysaccharides diffuse into the agar and form precipitation haloes with the specific antiserum. In LX, there is no such mechanism favoring a reaction of the capsular polysaccharide, and therefore positive reactions may be due to other cellular components, especially in noncapsulated strains in which the cell wall is more exposed. However, in the case of the Latex-MenB-positive autoagglutinable carrier strains the reactions seemed to be due to the polysaccharide, since they disappeared after absorption with group B but not with group A+C polysaccharide. This result may reflect a different sensitivity of the three methods so that strains that produce only small amounts of capsular polysaccharide are detected by LX but not by the other methods. The positive Latex-MenB reaction with the group 29 E carrier strain may, on the other hand, be due to the reaction of a non-polysaccharide component.

The latex reagents for serogrouping meningococci used throughout this study were prepared from horse or burro antisera obtained from J. B. Robbins. We have also prepared latex reagents from commercial meningococcal group A and C antisera (both Difco and Wellcome), and they have been equally satisfactory in routine use. We did not succeed in preparing a satisfactory Latex-MenB reagent from Difco antiserum, and the Latex-MenB reagent prepared from Wellcome antiserum, although specific for group B capsular polysaccharide, was less sensitive (detection limit 300 ng/ml) than the reagent used in this study (detection limit 25 ng/ml).

The great advantage of the LX method is its easy performance and interpretation. It is also inexpensive because only small quantities of antiserum are required for one determination. One can prepare about 150 ml of latex reagent from 1 ml of antiserum, and that amount of reagent is sufficient for about 3,000 tests. A drawback so far is that such latex reagents are not commercially available.

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