

Identification and Grouping of *Neisseria meningitidis* Directly on Agar Plates by Coagglutination with Specific Antibody-Coated Protein A-Containing Staphylococci

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It has been shown that *Neisseria meningitidis* can be grouped by coagglutination directly upon growth on sheep blood or chocolate agar plates. All positive reactions were group specific, and only a single colony was required for a positive reaction. There was variation seen in the effectiveness of commercial antisera in preparing sensitive reagents. Certain throat and sputum isolates of group Z organisms failed to react by coagglutination, although they would react directly with antiserum in whole-cell agglutination. This problem remains unresolved but may not be greatly significant in laboratory use of this method since group Z is rarely associated with disease.

The coagglutination test is based on strains of *Staphylococcus aureus* rich in protein A on their outer surfaces binding immunoglobulin G non-specifically through the Fc fraction (2, 3, 5), leaving specific Fab sites free to react with homologous antigen. The subsequent reaction of Fab with homologous antigen is made visible by clumping of *S. aureus*.

The use of coagglutination for grouping of *Neisseria meningitidis* has been previously reported (7). The tests were done on slides, either directly with cerebrospinal fluid or with suspensions of cultured organisms. Grouping of beta-hemolytic streptococci directly on sheep blood agar plates by coagglutination has also been reported (1). This study was designed to develop a coagglutination method for grouping *N. meningitidis* directly on agar plates which could replace counterimmunoelectrophoresis or slide-agglutination methods presently used.

MATERIALS AND METHODS

***S. aureus*.** The Cowan 1 strain (ATCC 12598) of *S. aureus* was provided by E. A. Edwards. The *S. aureus* preparation was that described by Kronvall (3) with the modifications described by Edwards and Larson (1).

Antisera to *N. meningitidis*. Commercial antisera to *N. meningitidis* groups A, B, C, D, X, Y, and Z were obtained from Difco Laboratories (Detroit, Mich.) and Burroughs Wellcome Co. (Research Triangle Park, N.C.). Additional group B and group Z antisera were supplied by Max Moody of Burroughs Wellcome. A group Z antiserum from the Center for Disease Control (CDC) was also used.

Sensitized reagents. Staphylococci were sensitized with antisera according to the methods of Kronvall (3) and Edwards and Larson (1). Two negative

control reagents were prepared with the staphylococci, one using normal rabbit serum and one using phosphate-buffered saline in place of antiserum. Reagents were thoroughly mixed on a Vortex mixer before used.

***N. meningitidis*.** Clinical isolates of *N. meningitidis* groups A (two strains), B (five strains), C (two strains), and Y (two strains) were obtained from specimens in our laboratory. These were identified by biochemical and slide-agglutination tests. The Indiana State Board of Health (ISBH) supplied one strain each of D (CDC strain KC688), X, and Z (patient isolates). Five additional strains of Z (strains KC662, D9206, D9210, D8334, D8954) were obtained from the CDC.

Other organisms. Six fresh clinical isolates of *Neisseria gonorrhoeae* were obtained from our clinical laboratories. Three strains of *Escherichia coli* with K1 antigen (O7:K1:NM 1722-72, ADO1:K1:NM 2906-76; O83:K1:H2 2549-77) were obtained from CDC.

Coagglutination on agar plates. *N. meningitidis* strains were grown on sheep blood agar plates for 18 to 24 h at 35°C in an atmosphere of 5% CO₂. Streaking on the plates was done in parallel lines about 0.5 inch (ca. 1.27 cm) apart so that up to 20 0.5-inch-diameter rings could be made on a plate with approximately the same amount of growth in each ring. This growth varied from confluent along a streak to a single colony per ring. Melted Vaspar (50:50, petrolatum and paraffin) was used to make rings around the growth and was applied to the plate with a no. 13 metal test-tube cap mounted on a handle. One drop of the reagent to be tested was placed in a ring, and the plate was gently rocked and observed under a dissecting microscope for agglutination for up to 8 min. Both control reagents were also tested on each plate in a similar fashion. A positive reaction usually appeared in 5 min or less. Up to 10 reagents could be tested on 1 plate simultaneously. Positive reactions were arbitrarily graded on a scale of 1+ to 4+, based on the amount of agglutination and the size of the clumps. A 1+ reaction was classified as a finely granular uniform agglutination. A 4+ reac-

tion showed large irregular clumps of agglutinated material. A positive test needed to show distinctly greater agglutination than the controls, and the test was invalid if controls showed more than 1+ agglutination.

N. meningitidis strains were also grown on chocolate agar and tested in the same manner as above. *N. gonorrhoeae* strains were grown on chocolate agar, and *E. coli* strains were grown on sheep blood agar and tested similarly.

RESULTS

Table 1 shows results obtained with *N. meningitidis* grown on sheep blood agar plates using Difco reagents, and Table 2 shows similar results using Burroughs Wellcome (BW) reagents. Both sets of reagents were initially tested versus one strain of each group. All of the positive reactions were group specific. In general, the reagents made with BW antisera gave stronger reactions. The BW reagents were therefore used in most of the testing of additional strains, as shown in Table 2.

Two problems were encountered in developing a complete set of seven group-specific and -sensitive reagents. First, it was difficult to get a satisfactory group B reagent. Two lots of group B antisera from Difco and two from BW proved inactive. It was only upon receipt of a new lot of group B antiserum from M. Moody (BW lot K3970) that an active reagent could be made. This reagent reacted strongly and specifically with all the group B strains tested (Table 2).

With only the patient isolate strain of group Z supplied by the ISBH, the group Z reagents also appeared to be inactive. However, upon receipt of five additional group Z strains from

CDC, it was found that one strain, KC662, reacted with the BW reagent, whereas the other four strains from CDC and the ISBH patient isolate strain did not. All six group Z strains were then tested with five group Z coagglutination reagents. These five coagglutination reagents were made with two lots of Difco antisera, two lots of BW antisera, and one antiserum from CDC. The results were the same for all five reagents: strain KC662 gave a positive reaction, and the other five strains gave negative reactions. In one case, strain D8334 versus CDC reagent, a questionable reaction of 1+ was observed.

The six strains of group Z were tested by whole-cell slide agglutination with two lots of BW and one lot of CDC antisera to group Z (Table 3). The results were not always consistent for the three antisera. However, none of these group Z strains appeared to have completely lost group Z antigen, since each reacted with at least one antiserum.

The coagglutination tests were repeated on organisms growing on chocolate agar to determine if the medium was affecting the antigen expression. Representative strains of groups A, B, C, D, X, Y, and all strains of group Z were tested. Agglutination could be seen on chocolate agar, although it was more difficult to read because the background provided less contrast. The results were the same as on the blood agar. All positive reactions were group specific and reached the same intensity in about the same time (± 1 min) as on blood agar. For group Z, again strain KC662 was positive, but the other five strains were negative.

TABLE 1. Coagglutination of *N. meningitidis* with reagents made with Difco antisera

Antigen ^a	Coagglutination group-specific reagents ^b						
	A (613770 ^c)	B (616216)	C (610600)	D (602683)	X (626898)	Y (614904)	Z (630863; 631448)
A PI-1 ^d	2+	—	—	—	—	—	—
B PI-1	—	—	—	—	—	—	—
C PI-1	—	—	3+	—	—	—	—
D CDC-KC688	—	—	—	1+	—	—	—
X PI-1	—	—	—	—	2+	—	—
Y PI-1	—	—	—	—	—	3+	—
Z PI-ISBH	—	—	—	—	—	—	—
Z CDC-KC662	ND ^e	ND	ND	ND	ND	ND	2+
Z CDC-D9206	ND	ND	ND	ND	ND	ND	—
Z CDC-D9210	ND	ND	ND	ND	ND	ND	—
Z CDC-D8334	ND	ND	ND	ND	ND	ND	—
Z CDC-D8954	ND	ND	ND	ND	ND	ND	—

^a *N. meningitidis* group and strain.

^b Normal rabbit serum and phosphate-buffered saline controls were all negative.

^c Antisera lot numbers.

^d PI, Patient isolate.

^e ND, Not done.

TABLE 2. Coagglutination of *N. meningitidis* with reagents made with BW antisera

Antigen ^a	Coagglutination group-specific reagents ^b						
	A (K2943 ^c)	B (K3970 ^d)	C (K2521)	D (K2030)	X (K1486)	Y (K1487)	Z (K1488; K3079)
A PI-1 ^e	4+	—	—	—	—	—	—
A PI-2	3+	—	—	—	—	—	—
B PI-1	—	4+	—	—	—	—	—
B PI-2	—	2+	—	—	—	—	—
B PI-3	—	2+	—	—	—	—	—
B PI-4	—	2+	—	—	—	—	—
B PI-5	—	3+	—	—	—	—	—
C PI-1	—	—	4+	—	—	—	—
C PI-2	—	—	4+	—	—	—	—
D CDC-KC688	—	—	—	4+	—	—	—
X PI-1	—	—	—	—	4+	—	—
Y PI-1	—	—	—	—	—	4+	—
Y PI-2	—	—	—	—	—	4+	—
Z PI-ISBH	—	—	—	—	—	—	—
Z CDC-KC662	—	—	—	—	—	—	4+
Z CDC-D9206	—	—	—	—	—	—	—
Z CDC-D9210	—	—	—	—	—	—	—
Z CDC-D8334	—	—	—	—	—	—	—
Z CDC-D8954	—	—	—	—	—	—	—

^a *N. meningitidis* group and strain.^b Normal rabbit serum and phosphate-buffered saline controls were all negative.^c Antisera lot numbers.^d Lot no. K2417 and K2717 were tested and did not react.^e PI, Patient isolate.TABLE 3. Whole-cell agglutination of *N. meningitidis* group Z

Antigen	Antisera		
	BW K1488 ^a	BW K3079 ^a	CDC
KC662	4+	4+	4+
D9206	2+	—	—
D9210	4+	4+	—
PI-ISBH	—	—	3+
D8334	3+	4+	2+
D8954	2+	3+	—

^a BW lot number.

To be useful in a clinical situation, this test should work on one or a few colonies rather than continuous growth. Plates were streaked for isolation of colonies, and single colonies of each group were tested. Both reaction strengths and reaction times for single colonies were not significantly different from those obtained with multiple colonies or confluent growth. None of the reactions were ambiguous. It was noted that continuous rocking of the plates was essential for development of the reactions in a minimal time.

The strains of *N. gonorrhoeae* and *E. coli* were tested for cross-reactions with the *N. meningitidis* BW coagglutination reagents. No positive reactions were seen between *N. gonorrhoeae* and any of the *N. meningitidis* reagents.

The strains of *E. coli* containing K1 antigen gave strong positive reactions with the *N. meningitidis* group B reagents but did not react with the other group reagents.

DISCUSSION

It has been demonstrated that coagglutination for grouping *N. meningitidis* can be done directly on colonies growing on agar plates, and only a single colony is required for a positive reaction. The test is easy to perform and does not require special equipment. In lieu of a dissecting microscope, results can usually be read with a good hand-held magnifying lens. Mixed cultures should not cause difficulty in coagglutination, as long as the correct organism is included in the test ring. If a spinal fluid or other specimen yields only one or two colonies, subculturing may have to be done before this test can be completed. Closely related *N. gonorrhoeae* showed no cross-reactions when tested with these reagents. However, cross-reaction between *N. meningitidis* group B reagent and *E. coli* with K1 antigen occurred as expected, since these organisms are known to share antigenic determinants (7). Gram stain and colonial morphology should resolve this cross-reaction when it occurs.

We have been unable to determine why five of the group Z strains did not react in coagglutination. Each of these strains did show whole-

cell agglutination with one or more antisera but failed to coagglutinate when the same sera were used in the coagglutination reagents. One reason may be differences in the expression of antigens and in the class of responsive antibodies. Whole-cell agglutination may be more dependent on immunoglobulin M, whereas immunoglobulin G is known to be the main class to bind to the protein A of *S. aureus* (2, 4). The strain KC662 that does react is the original group Z strain identified by Slaterus (8). The other five strains tested were from respiratory specimens such as throat swabs or sputums and were probably not causing disease at the time of isolation. Since group Z rarely seems to be a pathogen (6), non-coagglutinating group Z strains should not greatly impair the usefulness of this test.

The key to successful coagglutination is twofold; the availability of high-titered specific antisera, and staphylococcal preparations with good binding activity for antibody.

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