

Comparison of Commercial Diagnostic Tests for Identification of Serogroup Antigens of *Neisseria meningitidis*

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In the study that is described the sensitivities and specificities of three commercial tests and the standard Reference Laboratory test, used since 1961, to identify *Neisseria meningitidis* serogroups were compared. The tests marketed by Difco, Murex/Wellcome, and Sanofi/Pasteur showed overall sensitivities of 92, 95, and 100%, respectively, and specificities of 67, 88, and 82%, respectively. When limited to the common serogroups A, B, and C, the three tests yielded sensitivities of 93, 97, and 100%, respectively, and specificities of 98, 100, and 98%, respectively. However, determination of the uncommon serogroups X, W-135, Y, Z, and 29E with these tests is either unreliable or not possible.

Neisseria meningitidis is the predominant cause of bacterial meningitis in the Western world, with high morbidity. In addition meningococci can cause bacteremia and septicemia. Meningococci are divided into closely related but distinct serological groups, depending on the presence of capsular antigens. The recognized serogroups are A, B, C, D, W-135, X, Y, Z, 29E (occasionally called Z'), H, I, K, and L (1, 2, 5). For epidemiological studies, in order to develop vaccination strategies, it is important to assess which serogroups are circulating among the population. In The Netherlands Reference Laboratory for Bacterial Meningitis, a test to determine meningococcal serogroups was developed in 1961 (7). This serogrouping test with rabbit polyclonal antibodies elicited against the capsule polysaccharides of the different serogroups, including B, is done in an Ouchterlony assay. The polysaccharide antigen specific for meningococcus group B is only very slightly immunogenic. This is probably caused by immunotolerance resulting from cross-reactivity between antibodies against this polysaccharide and polysialic acid expressed on host neural cell adhesion molecules (4, 9). Therefore, it is difficult to obtain rabbit antibodies specifically directed against this polysaccharide. Generally, clinical microbiology laboratories are confined to a commercially available test, mostly in combination with tests for the identification of the other meningococcal serogroups. Here we report the results of a comparison of the sensitivities and specificities of three commercially available tests used to perform meningococcal serogroup determinations. In total, serogrouping of 72 *N. meningitidis* Reference Laboratory strains was done by the three commercial tests, while we were unaware of the serogroups previously assessed (Table 1). The isolates, which were stored on glass beads in glycerol bouillon (15% glycerol in Nutrient Broth no. 2; Oxoid CM 67, Unipath Ltd., Basingstoke, England) at -70°C , were plated onto chocolate agar plates. Besides *N. meningitidis* strains of serogroups A, B, C, W-135, X, Y, and 29E (Z'), six strains of the rare serogroups H (two isolates), I (two isolates), L (one isolate), and K (one isolate), and nine nongroupable isolates were included in the study. These latter 15 strains represent menin-

gococci that should yield a negative result by the three commercial tests. The test manufactured by Wellcome Diagnostics Limited (Dartford, England) uses rabbit polyclonal antibodies against serogroup A, C, W-135, X, Y, and Z antigens and a mouse monoclonal antibody against serogroup B antigen. Slide agglutination with these antibodies was used during serogrouping studies. The test manufactured by Difco Laboratories (Detroit, Mich.) consists of rabbit polyclonal antibodies specific for serogroups A, B, C, W-135, X, Y, Z, and 29E (Z'), and the slide agglutination assay was also used. The test marketed by Sanofi Diagnostics Pasteur (Marnes-la-Coquette, France) consists of latex particles coated with mouse monoclonal antibodies against serogroup B antigens or with rabbit polyclonal antibodies against the serogroup A and C antigens and the combined W-135 and Y antigens. These sensitized latex particles were used in an agglutination assay.

The determination of the serogroup antigen was performed according to the manufacturers' protocols. The results were compared with the results obtained by the standard Reference Laboratory method (7).

The number of concordant scores of each serogrouping test is provided in Table 1. Overall, the Difco, Murex/Wellcome, and Sanofi/Pasteur tests correctly scored the serogroups of 61, 67, and 67 of the 72 strains, respectively. One should keep in mind that serogroup 29E (Z') and serogroups X, Z, and 29E are not recognized by the Murex/Wellcome and Sanofi/Pasteur tests, respectively. These serogroups are included in the group of strains that should be negative by these tests, i.e., *N. meningitidis* strains of serogroups other than A, B, C, W-135, and Y. Table 2 summarizes the sensitivity and specificity of each of the three commercially available *N. meningitidis* serogrouping tests. Overall, the sensitivities were 92, 95, and 100% and the specificities were 67, 88, and 82% for the Difco, Murex/Wellcome, and Sanofi/Pasteur tests, respectively. *N. meningitidis* isolates of serogroups A, B, and C are the most common serogroups among patients with meningitis or septicemia. In total, the proportion of strains of these serogroups among isolates from Dutch patients with meningococcal disease was 98% for the past 5 years (6). When the tests were used to serogroup A, B, and C *N. meningitidis* isolates, the sensitivities of the three tests increased considerably to 93, 97, and 100% and the specificities increased to 98, 100, and 98% for the Difco, Murex/Wellcome, and Sanofi/Pasteur tests, respectively

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TABLE 1. Comparison of three commercially available tests for *N. meningitidis* serogrouping

Serogroup ^a	Total/concordant score serogrouped by:		
	Difco	Murex/Wellcome	Sanofi/Pasteur
A	10/9	10/10	10/9
B	10/9	10/10	10/10
C	10/9	10/9	10/10
W-135	6/5	6/5	6/6 ^b
X	6/5	6/5	— ^c
Y	9/7	9/9	9/9 ^b
Z	4/1	4/4	—
29E	2/2	—	—
Others ^d	15 ^d /14	17 ^e /15	27 ^e /23
Total	72/61	72/67	72/67

^a Determined by the standard assay of The Netherlands Reference Laboratory for Bacterial Meningitis.

^b The Sanofi/Pasteur test cannot distinguish between *N. meningitidis* isolates of serogroups Y and W-135.

^c —, the Sanofi/Pasteur and Murex/Wellcome tests do not contain antisera for the detection *N. meningitidis* serogroup X, Z, and 29E and serogroup 29E, respectively.

^d Serogroups H, I, K, and L and nonencapsulated isolates, which should be negative by the tests.

^e Serogroup 29E and serogroups X, Z, and 29E are also included for the Murex/Wellcome and Sanofi/Pasteur tests, respectively.

(Table 2). The overall positive predictive values were 87, 96, and 90% and the negative predictive values were 78, 83, and 100% for the Difco, Murex/Wellcome, and Sanofi/Pasteur tests, respectively (Table 3). Again, when only the antisera against *N. meningitidis* serogroups A, B, and C were used for serogrouping, the positive predictive values rose to 97, 100, and 97% and the negative predictive values rose to 98, 98, and 100% for the Difco, Murex/Wellcome, and Sanofi/Pasteur tests, respectively. For comparison, in particular the positive predictive values were significantly lower if only the antisera against serogroups W-135, X, Y, Z, and 29E would have been used for serogrouping: 77, 92, and 79% for the Difco, Murex/Wellcome, and Sanofi/Pasteur tests, respectively. Serogroups W-135, X, Y, and 29E are only rarely associated with disease, and patients with infections caused by isolates of these serogroups are often immunocompromised, mostly having one of the various complement deficiencies (3, 8). A reliable serogrouping test should be used to detect such patients. The antisera against serogroups W-135, X, Y, Z, and 29E in the

TABLE 2. Comparison of the sensitivities and specificities of three commercially available tests for *N. meningitidis* serogrouping

<i>N. meningitidis</i> serogrouping test	Sensitivity (%) of antisera against serogroups ^a :		Specificity (%) of antisera against serogroups ^a :	
	All	A, B, and C	All	A, B, and C
Difco	92	93	67	98
Murex/Wellcome	95	97	88	100
Sanofi/Pasteur	100	100	82	98

^a A positive reaction with a concordant score is considered true positive; positive reaction with a wrong score is considered false positive; a negative reaction is considered false negative when the test should detect the serogroup and true negative when the test is unable to detect the serogroup.

TABLE 3. Comparison of the positive and negative predictive values of three commercially available tests for *N. meningitidis* serogrouping

<i>N. meningitidis</i> serogrouping test	Positive predictive value (%) of antisera against serogroup ^a :		Negative predictive value (%) of antisera against serogroup ^a :	
	All	A, B, and C	All ^b	A, B, and C
Difco	87	97	78	98
Murex/Wellcome	96	100	83	98
Sanofi/Pasteur	90	97	100	100

^a A positive reaction with a concordant score is considered true positive; a positive reaction with a wrong score is considered false positive; a negative reaction is considered false negative when the test should detect the serogroup and true negative when the test is unable to detect the serogroup.

three serogrouping tests are less reliable, mainly because of false-positive scores, resulting in decreased positive predictive values. Meningococci of serogroups H, I, K, and L and non-groupable isolates have been isolated from carriers and have not been associated with disease. The antisera of the Sanofi/Pasteur test gave more false-positive results with these isolates than the other two tests, resulting in a considerably lower positive predictive value compared with those for the other two tests (Table 3).

In conclusion, the three commercial tests are reliable for serogrouping the common serogroups A, B, and C of *N. meningitidis*, but they are not appropriate for the detection of the uncommon serogroups X, W-135, Y, Z, and 29E.

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