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Serotypes and Subtypes of *Neisseria meningitidis*: Results of an International Study Comparing Sensitivities and Specificities of Monoclonal Antibodies

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An international study supported by the World Health Organization comparing monoclonal antibodies for serotyping and serosubtyping of *Neisseria meningitidis* strains was performed and the results were assessed in 1992. A collection of 6 serotype-specific (1, 2a, 2b, 4, 14, and 15) and 12 serosubtype-specific (P1.1, P1.2, P1.4, P1.5, P1.6, P1.7, P1.9, P1.10, P1.12, P1.14, P1.15, and P1.16) monoclonal antibodies was provided to 11 participating laboratories throughout the world. Monoclonal antibodies were tested on 85 *Neisseria meningitidis* strains with known reference results. Whole-cell enzyme-linked immunosorbent assay was used for analysis in 10 of 11 laboratories. The sensitivities and specificities of individual serotype- and subtype-specific monoclonal antibodies were evaluated. Differences in individual laboratories and with individual monoclonal antibodies were assessed. Relatively large differences in sensitivities were achieved in individual laboratories. On the contrary, the specificities remained at high levels in all laboratories. The sensitivities of serotype-specific monoclonal antibodies ranged from 72.0 to 100%. Individual serosubtype-specific monoclonal antibodies showed sensitivities ranging from 64.1 to 98.1%. The most frequent reason for the incorrect results obtained with the monoclonal antibodies were false-negative results. The collaborative study demonstrated that some monoclonal antibodies are not very sensitive. Another study to define the most suitable monoclonal antibodies is planned.

Neisseria meningitidis is one of the leading causes of bacterial meningitis (16). The development of a vaccine applicable in childhood appears to be the only means of decreasing the mortality and morbidity caused by meningococcal disease. Polysaccharide vaccines are available for serogroups A, C, Y, and W-135, albeit with the known shortcomings of polysaccharide vaccines, i.e., no induction of memory and poor immunogenicities in infants. The B polysaccharide is nonimmunogenic. Immunity against meningococcal disease was found to be strain dependent (7), i.e., dependent on the strain's group or type. Meningococci can be subdivided into serogroups on the basis of their capsular polysaccharide structures and into serotypes on the basis of their outer membrane proteins (OMPs) (6). The development of a group B meningococcal vaccine has resulted in field trials with OMP vaccines (3, 5). The bactericidal antibodies induced were found to be type specific (8). The determination of sero- and subtypes among meningococci on the basis of two different OMPs greatly improved after the introduction of monoclonal antibodies (13).

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In order to standardize serosubtyping we undertook a multicenter study in which 11 laboratories tested 85 meningococcal strains with the same lot of monoclonal antibodies in a blind manner. The outcome indicates that serosubtyping can be standardized. Blinded comparative studies between laboratories allows for the identification of suitable and less suitable monoclonal antibodies (specificity and sensitivity) and gives the opportunity to improve the performance of monoclonal antibodies in the laboratory.

MATERIALS AND METHODS

Monoclonal antibodies. The collection of serotype- and subtype-specific monoclonal antibodies, produced at the Rijksinstitut voor Volksgezondheid en Milieuhygiene (RIVM), Bilthoven, The Netherlands (Table 1), was provided to the 11 participating laboratories. Serotyping monoclonal antibodies were identified previously to react with the class 2/3 OMP, and the subtyping monoclonal antibodies were identified previously to react with the class 1 OMP.

***N. meningitidis* strains.** All participants received a collection of 85 *N. meningitidis* strains isolated in different countries. The 85 *N. meningitidis* strains were lyophilized and distributed by RIVM. The strains were recultivated according to the conditions used in each participating laboratory. The strains were declared to be *N. meningitidis* group B, and some laboratories verified this group identification.

Typing method. All except one of the laboratories used whole-cell enzyme-linked immunosorbent assay (ELISA) for serosubtyping (1, 13); the one exception used the dot blot technique. Laboratories reported their results as follows: positive, weakly positive, and nontypeable or nonsubtypeable. Two laboratories

TABLE 1. Monoclonal antibodies tested in the study

Type or subtype	Monoclonal	Immunoglobulin
Type		
1	MN3C6B	G2b
2a	MN2D3F	G2a
2b	MN2C3B	G2a
4	MN14G21.17	G2a
14	MN5C8C	G2a
15	MN15A14H6	G2a
Subtype		
P1.1	MN14C2.3	G2a
P1.2	MN16C13F4	G2a
P1.4	MN20B9.34	G2a
P1.5	MN22A19.9	G2a
P1.6	MN19D6.13	G3
P1.7	MN14C11.6	G2a
P1.9	MN5A10F	G2a
P1.10	MN20F4.17	G2b
P1.12	MN21A7.10	G3
P1.14	MN21G3.17	G3
P1.15	MN3C5C	G3
P1.16	MN5C11G	G2b

reported a part of their results as optical density values and were asked to sort these values into the same categories used by the other participating laboratories.

Statistical analysis. The results were processed with a Macintosh personal computer on FILE MAKER and CRICKET GRAPH 1.32 software. The sender of the pretested 85 *N. meningitidis* strains was asked to submit the reference results for the calculation of sensitivities and specificities (12), that is, the results found previously with the same monoclonal antibodies evaluated in the present study, albeit with different lots.

Weakly positive results were assessed in two ways: as positive and negative. Differences in both evaluations were analyzed. Because a class 1 OMP has two independent subtype epitopes, the reported positive results were assessed without consideration of their double appearance in subtype-positive results. Some of the *N. meningitidis* strains failed to grow in different laboratories, and statistical analysis was performed with the consideration of the number of strains tested by the participating laboratories.

This report includes data from 10 laboratories only, because the results from 1 laboratory included an extremely high number of false-positive results with multiple positive results (for nearly all monoclonal antibodies tested) which were not comparable to the results of the other laboratories (and seem to have been caused by some technical error). Laboratories were numbered by chance, and the results are presented under those numbers.

RESULTS

Sensitivity and specificity differences between laboratories. Relatively large differences in sensitivities were achieved between different laboratories, ranging from 53.3 to 100% for type-specific monoclonal antibodies (Fig. 1) and from 58.0 to 97.0% for subtype-specific monoclonal antibodies (Fig. 2).

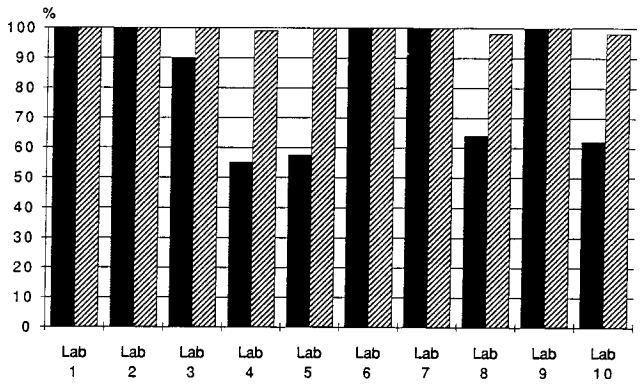

FIG. 1. Sensitivity (■) and specificity (▨) of type-specific monoclonal antibodies in 10 laboratories (85 *N. meningitidis* strains).

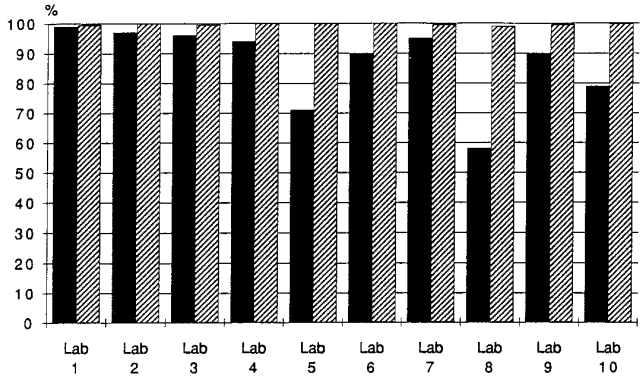

FIG. 2. Sensitivity (■) and specificity (▨) of type-specific monoclonal antibodies in 10 laboratories (85 *N. meningitidis* strains).

Contrary to these variations in the sensitivities between different laboratories, the specificities remained at high levels in all laboratories, ranging from 98.5 to 100% for type-specific monoclonal antibodies (Fig. 1) and from 99.4 to 99.9% for subtype-specific monoclonal antibodies (Fig. 2). Consequently, the performance of the individual laboratories influenced the final values for individual monoclonal antibodies.

Differences in type-specific monoclonal antibodies. Individual type-specific monoclonal antibodies showed sensitivities ranging from 72.0 to 100% and specificities ranging from 96.8 to 99.9% (Table 2). The highest degree of sensitivity was achieved with monoclonal antibody type 2a; the lowest was achieved with monoclonal antibody type 4 and then types 1 and 15. The most frequent reason for incorrect results for monoclonal antibody 4 was false negativity.

Differences in subtype-specific monoclonal antibodies. Individual subtype-specific monoclonal antibodies showed sensitivities ranging from 64.1 to 98.1% and specificities ranging from 98.9 to 100% (Table 3). The highest degree of sensitivity was achieved by monoclonal antibody subtype P1.15; the lowest degree of sensitivity was found with monoclonal antibody subtype P1.6 and then subtypes P1.10 and P1.5. The most frequent reason for incorrect results with these monoclonal antibodies was again false negativity.

Multiple positive results. In the reference results for the 85 *N. meningitidis* strains, a double positivity of subtype-specific monoclonal antibodies was reported for 24 strains. The combinations of double positivity revealed by participants were

TABLE 2. Results for individual type-specific monoclonal antibodies tested in 10 laboratories with 85 *N. meningitidis* strains

Monoclonal antibody	No. of reported results				Sensitivity (%)	Specificity (%)
	True positive	True negative	False positive	False negative		
1	20	780	1	6	76.9	99.9
2a	67	737	3	0	100.0	99.6
2b	9	796	1	1	90.0	99.9
4	113	643	21	44	72.0	96.8
14	88	709	4	6	93.6	99.4
15	64	723	5	15	81.0	99.3
Total	361	4,389	35	72	83.4	99.2

TABLE 3. Results for individual subtype-specific monoclonal antibodies tested in 10 laboratories with 85 *N. meningitidis* strains

Monoclonal antibody	No. of reported results				Sensitivity (%)	Specificity (%)
	True positive	True negative	False positive	False negative		
P1.1	73	922	0	8	90.1	100.0
P1.2	123	873	10	6	95.3	98.9
P1.4	26	981	0	2	92.8	100.0
P1.5	117	779	4	30	79.6	99.5
P1.6	50	943	1	28	64.1	99.9
P1.7	91	907	1	16	85.0	99.9
P1.9	93	914	1	3	96.9	99.9
P1.10	13	993	1	6	68.4	99.9
P1.12	45	957	2	5	90.0	99.8
P1.14	34	969	11	2	94.4	98.9
P1.15	53	949	7	1	98.1	99.3
P1.16	50	952	3	6	89.3	99.7
Total	768	11,139	41	113	87.3	99.6

found in 83.2% of the reference results, while double positivity different from the reference results was very rare (data not shown).

DISCUSSION

The blinded collaborative study on meningococcal serosubtyping described here was undertaken to establish the specificities and sensitivities of the available monoclonal antibodies and the laboratory-to-laboratory variability. The collaborative study has demonstrated that some monoclonal antibodies against meningococcal OMPs (serotypes 4, 1, and 15 and subtypes P1.6, P1.10, and P1.15) are not very sensitive. Another study to define the most suitable monoclonal antibodies is planned. Some laboratories reported a small number of the results as weakly positive. The analysis of these results, in which weakly positive results were scored either as positive or as negative, revealed some nonsignificant differences in sensitivity, especially with regard to subtype-specific monoclonal antibodies.

The biggest differences in the results obtained by the various laboratories were with the monoclonal antibodies with the lowest degrees of sensitivity. The most frequent reason for the incorrect results obtained with the monoclonal antibodies was false negativity, which was most often reported by the same laboratories. These findings illustrate that various laboratory practices have an influence on the results, and correct performance of the recommended typing method is needed. Further studies that involve the laboratories that achieved good sensitivities in the study presented here would provide a better possibility of comparing the qualities of the monoclonal antibodies tested.

It was agreed during a meeting on meningococcal typing in June 1992 in Atlanta, Ga. (11), that the British National Control Laboratory (National Institute for Biological Standards and Control, Hertfordshire, United Kingdom) will establish a hybridoma collection and produce monoclonal antibodies. In collaboration with RIVM new lots of the serosubtyping kit will be distributed.

In some countries a large portion of nontypeable and nonsubtypeable *N. meningitidis* remains, despite the availability of commonly used monoclonal antibodies and typing techniques. For this reason the preparation of new monoclonal antibodies from the strains of these countries would be desirable.

We plan to accomplish a global overview of meningococcal serotypes and serosubtypes when a good collection of suitable monoclonal antibodies is available to compare the disbutions of serosubtypes with the distributions monitored previously (14). Until now, the availability of different kits of various monoclonal antibodies has had an overwhelming influence on practically all serosubtyping studies, and the results of those studies are not fully comparable (2, 4, 9, 10, 15, 16).

Further studies could clarify the influences of, for example, cultivation of *N. meningitidis*, antigen preparation, and storage of coated microtiter plates.

The strains included in the present study were declared to be *N. meningitidis* group B. In several participating laboratories different serogroups were revealed as follows: group A (1 strain), group C (3 strains), and group W-135 (1 strain). These differences were not considered to be significant.

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