Serological Specificities of Murine Hybridoma Monoclonal Antibodies against *Neisseria meningitidis* Serogroups B, C, Y, and W135 and Evaluation of Their Usefulness as Serogrouping Reagents by Indirect Whole-Cell Enzyme-Linked Immunosorbent Assay

Raymond S. W. Tsang¹* and Wendell D. Zollinger²

1Laboratory for Pathogenic Neisseria and Vaccine Preventable Bacterial Diseases, National Microbiology Laboratory, Health Canada, Winnipeg, Manitoba, Canada, ¹ and Walter Reed Army Institute of Research, Washington, D.C. ²

Received 12 July 2004/Returned for modification 30 September 2004/Accepted 9 November 2004

Molecular hybridoma monoclonal antibodies (MAbs) were produced against the capsular antigens of serogroups B, C, Y, and W135 meningococci. Each serogroup-specific MAb reacted with the extracted capsular polysaccharide from its homologous serogroup only and did not react with capsules from the other three serogroups. The application of these MAbs for serogroup identification of meningococci was demonstrated by their abilities to correctly identify 183 clinical isolates of 185 meningococci recovered from individual invasive meningococcal disease (IMD) patients during routine surveillance in 2002. The remaining two meningococci were identified by PCR grouping as C in one case and Y in another, but neither isolate was positive by bacterial agglutination using rabbit antiserum or by enzyme-linked immunosorbent assay using MAbs. The specificities of the anti-Y and anti-W135 MAbs were further assessed by tests with 37 serogroup W135 and 106 serogroup Y meningococci recovered from IMD cases during 1999 to 2001 and 2003. All 143 meningococci except one serogroup Y isolate were correctly identified by positive reactions with the corresponding MAbs that identified their homologous serogroups. The single serogroup Y isolate was received as nonagglutinable and tested as negative with both rabbit anti-Y antiserum and anti-Y MAb but was positive for the serogroup Y-specific *sidD* gene. The advantage of using MAbs for serogrouping of meningococci is discussed.

*Neisseria meningitidis* remains a significant human pathogen and a leading cause of meningitis and septicemia in adults as well as in children. Besides causing a significant case fatality rate of about 9 to 12% (27), complications and postinfectious sequelae are also common (12, 14). Of the 13 known serogroups, classified based on serological specificities of their capsular antigens (30), most (>90%) diseases are caused by organisms belonging to serogroups A, B, C, Y, and W135 (21, 24). In Canada as well as in the United States, diseases caused by serogroup A organisms are no longer endemic, and most endemic as well as epidemic diseases are now caused by organisms belonging to serogroups B, C, Y, and W135 (26, 28).

Serogrouping of *N. meningitidis* (or meningococci) is important because the disease caused by some serogroups can be prevented by active immunization. Therefore, in outbreak situations as well as in routine surveillance of this disease, results of serogroup determination may lead to decisions on the public health intervention measures to be taken. Serogroup determination may also help in understanding the changing epidemiology of meningococcal disease (26, 28). Since serogroup classification is based on unique antigens present on the capsules of meningococci, traditionally serogrouping is done by detection of these antigens with specific antiserum using the simple bacterial agglutination test. Most serogrouping antisera are produced in rabbits, but antiserum towards carbohydrate antigens are usually of low titer, especially in the case of antisera to serogroup B meningococci, because of the molecular mimicry between the serogroup B capsule and the tissue antigen of neuron cell adhesion molecule (20). Therefore, antiseras are usually used without cross-absorption with related serogroups of meningococci to remove antibodies to the common outer membrane protein antigens, and specificity of such polyclonal antiseras remains an issue. For example, in a study to compare commercial serogrouping antisera for the identification of serogroup antigens of *N. meningitidis*, it was found that such reagents had good sensitivities and specificities for detection of the more common serogroups A, B, and C but were not satisfactory for determination of the uncommon serogroups, including Y and W135 (29).

Besides bacterial agglutination, other methods have been proposed for serogrouping of meningococci, and these include an antiserum agar method (2, 3, 9, 10), coagglutination with serogroup-specific antibodies coated onto protein A-rich staphylococci (32), latex agglutination (16), membrane-based dot immunoassay (23), and enzyme-linked immunosorbent assay (ELISA) (31). Also, monoclonal antibodies (MAbs) to the serogroup-specific antigens of *N. meningitidis* serogroup A, B, and C bacteria have also been described (5, 13, 22). Detection of the serogrouping antibodies by antibodies may sometimes give negative results because of down-regulation of the capsule synthesis genes, and this is especially common among isolates recovered from carriers (8). Therefore, a number of molecular methods have also been proposed for determination of the serogroup nature of meningococci. Most of these molecular methods are PCR-based assays designed to detect either the

---

¹ Corresponding author. Mailing address: National Microbiology Laboratory, 1015 Arlington St., Winnipeg, Manitoba, Canada R3E 3R2. Phone: (204) 789-6020. Fax: (204) 789-2018. E-mail: rtsang@hc-sc.gc.ca.
serogroup-specific siaD gene in serogroups B, C, Y, and W135 (6, 7), or the serogroup A capsule-specific myn gene (11), or specific regions towards the 5’ end of the cra4 genes for serogroups 29E, X, and Z (4). Real-time PCR (19, 25) and nucleotide sequence analysis (17) have been proposed for identification of serogroup Y and W135 meningococci.

In this paper, we describe the serological specificities of murine hybridoma MAbs against the capsular antigens of four common serogroups of meningococci in North America (B, C, Y, and W135), and we report their usefulness as serogrouping reagents.

**MATERIALS AND METHODS**

*Neisseria meningitidis* strains and biochemical identification. Isolates of *N. meningitidis* from invasive meningococcal disease (IMD) cases, defined as a patient presenting with symptoms compatible with meningococcal disease such as meningitis, septicemia, petechiae-purpura, and fever, were selected for this study. They were submitted to Health Canada’s National Microbiology Laboratory by provincial and territorial public health laboratories across Canada for national surveillance of IMD (28). Their identities were confirmed by standard biochemical tests. A total of 185 isolates collected from individual IMD cases in 2002 were included for this study. These 185 isolates included 77 serogroup B, 66 serogroup C, 29 serogroup Y, 6 serogroup W135, and 7 isolates that were nonserogroupable. Because of the small number of serogroup Y and W135 isolates collected in 1 year, additional serogroup Y (a total of 106 isolates) and serogroup W135 (a total of 37 isolates) meningococci collected within the years 1999 to 2001 and 2003 were also used in this study. Production of polyclonal rabbit antiserum and murine hybridoma MAbs to serogroup antigens of *N. meningitidis*. Rabbit antisera to the capsular polysaccharide antigens of serogroups A, B, C, W135, X, Y, Z, and 29E were produced according to the method described by Ashton et al. (2). Albino rabbits were immunized by intravenous injections of a viable suspension of the bacteria adjusted to a cell density equivalent to a turbidity of a McFarland number 3 standard. The dosage and schedule of the immunizations involved 3 injections per week given on alternate days with 0.2, 0.2, and 0.3 ml for week 1, followed by three 0.5 ml injections in week 2 and 1.0, 1.0, and 1.5 ml for week 3.

Murine MAbs to the capsular antigens of serogroups B, C, W135, and Y were produced by the standard hybridoma method of fusing immune splenocytes from BALB/c mice with myeloma cells, as described by Kohler and Milstein (15) and Mandrell and Zoller (18). Mice were immunized with live bacterial whole cells suspended in sterile phosphate-buffered saline (PBS) to give a cell density (optical density at 600 nm) of about 0.25, given by both subcutaneous (0.05 ml) and intravenous (0.05 ml) routes twice either 1 or 2 months apart. Isotypes of the MAbs were determined with the mouse MAb isotyping kit (Roche Diagnostics, Laval, Quebec, Canada).

**Bacterial agglutination.** The bacterial agglutination test to determine the serogroup identity of meningococci was done as described by Ashton et al. (2).

**ELISA.** Serogrouping by MAbs was done by an indirect whole-cell ELISA, which was a modification of the method of Abdillahi and Poolman (1) described for typing of *N. meningitidis*. Briefly, to prepare the ELISA antigens for testing, an overnight growth of *N. meningitidis* was added for color development at room temperature. ELISA read-

![Image](https://via.placeholder.com/150)

**TABLE 1. Antigenic specificities of antimeningococcal MAbs against extracted capsular polysaccharides by indirect ELISA.**

| MAb and dilution | Mean* ELISA OD against polysaccharides extracted from serogroup: |
|------------------|---------------------------------------------------------------|
|                  | B   | C   | Y   | W135 |
| Anti-B, 2-2-B    |     |     |     |      |
| 1:200            | 2.371^b | 0.003 | 0.002 | 0.003 |
| 1:500            | 1.244 | 0.001 | 0.000 | 0.001 |
| Anti-C, 4-2-C    |     |     |     |      |
| 1:500            | 0.005 | 3.919 | 0.001 | 0.002 |
| 1:1,500          | 0.000 | 3.884 | 0.002 | 0.001 |
| Anti-Y, 5-1-Y    |     |     |     |      |
| 1:500            | 0.000 | 0.002 | 3.041 | 0.002 |
| 1:5,000          | 0.001 | 0.001 | 0.344 | 0.001 |
| Anti-W135, 6G9-7 |     |     |     |      |
| 1:500            | 0.002 | 0.002 | 0.001 | 3.154 |
| 1:5,000          | 0.002 | 0.002 | 0.000 | 0.557 |

* Mean of duplicate determinations at 30 min.

^b Bold values indicate positive results for binding of serogroup-specific capsular polysaccharide.
Capsule polysaccharide antigens were coated at a concentration of 10 μg per ml in PBS at 4°C overnight. The rest of the procedure was similar to the indirect whole-cell ELISA method described above.

**Grouping by PCR detection of serogroup-specific siaD gene.** Grouping of meningococci by PCR amplification and hybridization (PCR-ELISA) to detect the siaD genes specific to serogroups B, C, Y, and W135 were done essentially as described by Borrow et al. (6, 7).

**RESULTS**

**Specificities of murine hybridoma MAbs.** Four MAbs were selected for this study. They were 2-2-B (IgM/κ) for serogroup B, 4-2-C (IgG3/κ) for serogroup C, 5-2-Y (IgM/κ) for serogroup Y, and 7-1-W (IgG3/κ) for serogroup W135. All four MAbs were found to react with extracted capsules from the homologous serogroup but did not react with related polysaccharides from the heterologous serogroups (Table 1). When each of the four MAbs was tested against all 13 known serogroups of meningococci by indirect whole-cell ELISA, the anti-B and anti-C MAbs were found to be highly specific and showed strong reactions with meningococci of the homologous serogroups only, without any evidence of cross-reactions with cells of the heterologous serogroups. However, the anti-Y (5-1-Y) and anti-W135 (7-1-W) MAbs showed traces of cross-reactions with cells of other serogroups besides reacting strongly with their homologous serogroups. The anti-Y MAb was found to give weak reactions with cells of serogroups D, 29E, H, I, K, and L meningococci, while the anti-W135 MAb was found to react weakly with cells of serogroups D and L. In both cases, reactions of the MAbs with cells of their homologous serogroups gave ELISA ODs much higher than ODs observed in the cross-reactions (Table 2).

**Evaluation of MAbs as reagents for serogrouping of meningococci.** A total of 178 strains out of a total of 185 meningococci collected from IMD cases in 2002 gave concordant results when their serogroups were determined by using two different kinds of serogrouping antibodies and two different serological methods (rabbit polyclonal antiserum used in a slide agglutination test versus mouse MAbs used in an ELISA) (Table 3). Five isolates that were nongroupable (because they were either nonagglutinable or polyagglutinable) with rabbit antiserum were identified by MAbs to be serogroup B (one isolate) and serogroup C (four isolates). Two isolates were found to be negative for the presence of serogroup antigens regardless of whether rabbit antiserum or mouse MAbs were used. These two isolates were identified by PCR grouping as C (one isolate) and Y (one isolate).

The reactions of 37 serogroup W135 and 106 serogroup Y isolates with the anti-Y and anti-W135 MAbs are summarized in Table 4. All 37 serogroup W135 isolates gave clear-cut strong reactions with the anti-W135 MAb (mean ELISA OD, 1.838; standard deviation, [SD], 0.835; range, 0.939 to 3.916) and did not react with the anti-Y MAb (mean ELISA OD, 0.039; SD, 0.02; range, 0.008 to 0.089). A total of 105 out of 106 serogroup Y isolates were detected by the anti-Y MAb (mean ELISA OD, 0.711; SD, 0.314; range, 0.212 to 1.848). Their reactions with the anti-W135 MAbs gave a mean ELISA OD of 0.025 with a range of 0.008 to 0.054 and an SD of 0.009. The serogroup Y isolate that did not react with the anti-Y MAb was submitted to our laboratory as a nonagglutinable isolate, and it was identified as serogroup Y by siaD PCR-ELISA.

**DISCUSSION**

The specificities of four murine hybridoma MAbs to serogroup B, C, Y, and W135 meningococci were confirmed by their reactions towards the extracted capsular antigens from

### TABLE 3. Comparison of polyclonal versus monoclonal antibodies for serogroup identification of serogroup Y and W135 meningococci

| Rabbit antiserum | No. of isolates | MA | Positive reaction ODs | Negative reaction ODs |
|------------------|----------------|----|-----------------------|-----------------------|
|                  |                | B  | Mean      | SD     | Range            | Mean      | SD     | Range            |
|                  |                | C  | 1.268     | 0.385  | 0.326–2.043      | 0.026     | 0.021  | 0.00–0.123       |
|                  |                | Y  | 1.782     | 0.560  | 0.617–2.615      | 0.014     | 0.019  | 0.00–0.076       |
|                  |                | W135 | 1.803   | 0.929  | 0.722–2.912      | 0.024     | 0.028  | 0.00–0.095       |
|                  |                | Nongroupable | 1     | 1.581   | NA*               | 0.057     | NA     | NA               |
|                  |                | Nongroupable | 4     | 2.250   | 0.826 1.236–3.113 | 0.040     | 0.024  | 0.003–0.088      |

| No. identified by MAbs | No. negative with MAbs | Mean | SD | Range |
|------------------------|------------------------|------|----|-------|
|                         |                        | 1.838| 0.835| 0.939–3.916 |
|                         |                        | 0.039| 0.020| 0.008–0.089 |

*In-house-produced antiserum and serogroup determined by bacterial agglutination method.

Serogroup determined by indirect whole-cell ELISA.

Grouping confirmed by PCR detection of serogroup-specific siaD genes.

Identified by PCR detection of serogroup-specific siaD genes as C (one isolate) and Y (one isolate).

NA, not applicable.

### TABLE 4. Specificity testing of anti-Y and anti-W135 MAbs against N. meningitidis serogroup Y and W135 isolates obtained from Canadian IMD cases in 2003 and 1999 to 2001

| Serogroup | No. of strains | No. identified by MAbs | No. negative with MAbs | Positive reaction ODs | Negative reaction ODs |
|-----------|----------------|------------------------|-----------------------|-----------------------|-----------------------|
| W135      | 37             | 37                     | 0                     | 1.838                 | 0.039                 |
| Y         | 106            | 105                    | 1                     | 0.711                 | 0.025                 |

* Serogroups identified by bacterial agglutination with rabbit antiserum (2).

The single isolate was identified as group Y by PCR detection of the serogroup Y-specific siaD gene.
homologous serogroups of meningococci without any traces of cross-reactions with the polysaccharides from the other three serogroups. This was confirmed with bacterial whole cells as antigens in an indirect whole-cell ELISA (Table 2). The small amount of reactivity found for the anti-Y and anti-W135 MAbs with serogroup D, 29E, H, I, K, and L meningococci cannot be readily explained from our current knowledge about the capsular and/or other surface structures of these serogroups of meningococci. Nevertheless, these minor cross-reactions should not pose any problem in routine use of the anti-Y and anti-W135 MAbs, since serogroup D, H, I, K, and L meningococci are rare in North America and their reactions with the anti-Y and anti-W135 MAbs gave much lower ELISA ODs (in the range of 0.110 to 0.215) compared to the reactions of serogroup Y meningococci reacting with anti-Y MAb (ELISA OD, 1.987) and serogroup W135 meningococci reacting with anti-W135 MAb (ELISA OD, 2.973).

With regards to serogroup 29E meningococci, a specific MAb is also available in our laboratory, and preliminary testing has shown that this antibody is specific for its homologous serogroup [W. D. Zollinger, unpublished data]. Besides reacting with the serogroup B meningococci, the anti-serogroup B MAb 2-2-B also reacted with K1-positive ing with the serogroup B meningococci, the anti-serogroup B anti-W135 MAb (ELISA OD, 2.973). Serogroup Y meningococci reacting with anti-Y MAb (ELISA OD, 0.110 to 0.215) compared to the reactions of anti-Y and anti-W135 MAbs gave much lower ELISA ODs (in the range of 0.110 to 0.215) compared to the reactions of serogroup Y meningococci reacting with anti-Y MAb (ELISA OD, 1.987) and serogroup W135 meningococci reacting with anti-W135 MAb (ELISA OD, 2.973).

Serological cross-reaction between serogroups Y and W135 has been described, and it is usually ascribed to the antigenic similarities of their capsular polysaccharides (8, 9). For example, it has been observed with the antiserum agar method that serogroup Y isolates produce immunoprecipitation lines with not only anti-Y antiserum but also with anti-W135 antiserum (9, 10). We have also observed isolates showing polyagglutinations in both anti-Y and anti-W135 meningococci (National Microbiology Laboratory, unpublished observations). We therefore examined the reactions of anti-Y and anti-W135 MAbs against most of the serogroup Y and W135 meningococci collected in our laboratory from IMD cases over the last few years. Testing with 106 serogroup Y isolates and 37 serogroup W135 bacteria confirmed the specificities of these two MAbs (Table 4).

The routine application of MAbs in serogroup determinations was shown by the results presented in Table 3. Five isolates that were either nonagglutinable or polyagglutinatable in the bacterial agglutination test with rabbit serogrouping antisera were identified by MAbs, thus showing an advantage of the ELISA method over the bacterial agglutination test. ELISA or dot blot immunoassay is routinely used in meningococcal reference laboratories for identification of serotype and serosubtype antigens of meningococci (1). Therefore, serogrouping with MAbs using the ELISA method does not require a methodology that is not already being used. A further advantage of the ELISA method over the bacterial agglutination test is the fact that isolates that tend to autoagglutinate can still be grouped, since the binding of MAbs to the meningococcal cells is detected by an enzyme label instead of relying on the bacterial agglutination of the antigen-antibody binding reaction. Finally, detection of the presence of the capsular antigens confirmed the expression of the antigens, while detection of a specific siaD gene does not allow one to predict whether capsule will be expressed or not.

Besides using the MAbs in a whole-cell ELISA, we have also produced high titers of these antibodies under tissue culture conditions using Integra flasks (Integra Biosciences Inc., Zurich, Switzerland), and these high-titer antibodies (in the range of over 1:100,000 by ELISA detection) allow meningococci to be agglutinated in a specific fashion, just like when using poly-}

**ACKNOWLEDGMENTS**

We thank Dennis Law, Averil Henderson, and Lisa Schmidt for their assistance in this work. We also acknowledge the contributions of the Directors and staff of the provincial and territorial public health laboratories to the National Surveillance Program in submitting IMD isolates for antigenic and genetic characterization.

We thank Health Canada’s Genomics R&D Fund for financial assistance.

**REFERENCES**

1. Abdillahi, H., and J. T. Poolman. 1987. Whole cell ELISA for typing Neisseria meningitidis with monoclonal antibodies. FEMS Microbiol. Lett. 48: 367–371.
2. Ashton, F. E., A. Ryan, B. B. Diena, and C. E. Frasch. 1979. Evaluation of the antiserum agar method for the serogroup identification of Neisseria meningitidis. Can. J. Microbiol. 25:784–787.
3. Ashton, F. E., A. Ryan, and B. B. Diena. 1980. Improved antiserum agar method for the serogroup differentiation of Neisseria meningitidis Y and W135. Can. J. Microbiol. 26:630–632.
4. Bennett, D. E., R. M. Mulhall, and M. T. Caferkey. 2004. PCR-based assay for detection of Neisseria meningitidis capsular serogroups 29E, X, and Z. J. Clin. Microbiol. 42:1764–1765.
5. Bitter-Suermann, D., and J. Roth. 1987. Monoclonal antibodies to polysialic acid reveal epitope sharing between invasive pathogenic bacteria, differentiating cells and tumor cells. Immunol. Res. 6:225–237.
6. Borrow, R., H. Claus, M. Guiver, L. Smart, E. B. Kaczmarski, M. Frosch, and A. J. Fox. 1997. Non-culture diagnosis and serogroup determination of meningococcal B and C infection by a sialidase assay (siaD PCR). Epidemiol. Infect. 118:111–117.
7. Borrow, R., H. Claus, U. Chaudhry, M. Guiver, K. B. Kaczmarski, M. Frosch, and A. J. Fox. 1998. siaD PCR ELISA for confirmation and identification of serogroup Y and W135 meningococcal disease. FEMS Microbiol. Lett. 159:209–214.
8. Claus, H., M. C. J. Maiden, R. Maag, M. Frosch, and U. Vogel. 2002. Many carried meningococci lack the genes required for capsule synthesis and transport. Microbiology 148:1813–1819.
9. Craven, D. E., C. E. Frasch, J. R. Robbins, and H. A. Feldman. 1978. Serogroup identification of Neisseria meningitidis: comparison of an antisera agar method with bacterial slide agglutination. J. Clin. Microbiol. 4:410–414.
10. Craven, D. E., and C. E. Frasch. 1979. Serogroup identification of meningococci by a modified antiserum agar method. J. Clin. Microbiol. 9:478–484.
11. Diggle, M., K. Smith, E. Girvan, and S. Clarke. 2003. Evaluation of a fluorescence-based PCR method for identification of serogroup A meningococci. J. Clin. Microbiol. 41:1766–1768.
12. Edwards, M. S., and C. J. Baker. 1981. Complications and sequelae of meningococcal infections in children. J. Pediatr. 99:540–545.
13. Hurpin, C. M., E. D. Carosella, and P. A. Cazenave. 1992. Bactericidal activity of two IgG2a murine monoclonal antibodies with distinct fine specificities for group B Neisseria meningitidis capsular polysaccharide. Hybridoma 11:677–687.
14. Kirsch, E. A., R. P. Barton, L. Kitchen, and B. P. Giroir. 1996. Pathophysiology, treatment and outcome of meningococemia: a review and recent experience. Pediatr. Infect. Dis. J. 15:567–578.
15. Kohler, G., and C. Milstein. 1975. Continuous culture of fused cells secreting antibody of predefined specificity. Nature (London) 256:495–497.
16. Kuipers, B., G. van den Dobbelsteen, E. Wedege, and L. Van Alphen. 2001. Serological characterization. Methods Mol. Med. 67:131–145.
17. Lewis, C., and S. C. Clarke. 2003. Identification of Neisseria meningitidis serogroups Y and W135 by siaD nucleotide sequence analysis. J. Clin. Microbiol. 41:2697–2699.
18. Mandrell, R. E., and W. D. Zollinger. 1982. Measurement of antibodies to meningococcal group B polysaccharide: low avidity binding and equilibrium binding constants. J. Immunol. 129:2172–2178.
19. Mothershed, E. A., C. T. Sacchi, A. M. Whitney, G. A. Barnett, G. W. Ajello, S. Schmink, L. W. Mayer, M. Phelan, T. H. Taylor, Jr., S. A. Bernhardt, N. E. Rosenstein, and T. Popovic. 2004. Use of real-time PCR to resolve slide agglutination discrepancies in serogroup identification of Neisseria meningitidis. J. Clin. Microbiol. 42:320–328.
20. Muhlenhoff, M., M. Eckhardt, and R. Gerardy-Schahn. 1998. Polysialic acid: three dimensional structure, biosynthesis and function. Curr. Opin. Struct. Biol. 8:558–564.
21. Munford, R. S. 2001. Meningococcal infections: disease caused by gram-negative bacteria, p. 927–931. In E. Braunwald, A. S. Fauci, D. L. Kasper, S. L. Hauser, D. L. Longo, and J. L. Jameson (ed.), Harrison’s principles of internal medicine, 15th ed. McGraw-Hill Medical Publishing Division, New York, N.Y.
22. Nato, F., J. C. Mazie, J. M. Fournier, B. Slizewicz, N. Sagot, M. Guibourdenche, D. Postic, and J. Y. Riou. 1991. Production of polyclonal and monoclonal antibodies against group A, B, and C capsular polysaccharides of Neisseria meningitidis and preparation of latex reagents. J. Clin. Microbiol. 29:1447–1452.
23. Oprandy, J. J., and J. E. Sippel. 1989. Evaluation of performance parameters of a membrane-based dot immunoassay for meningococcal polysaccharide. J. Clin. Microbiol. 27:74–77.
24. Peltola, H. 1983. Meningococcal disease: still with us. Rev. Infect. Dis. 5:71–91.
25. Probert, W. S., S. L. Bystrum, S. Khashe, K. N. Schrader, and J. D. Wong. 2002. 5’ exonuclease assay for detection of serogroup Y Neisseria meningitidis. J. Clin. Microbiol. 40:4325–4328.
26. Rosenstein, N. E., B. A. Perkins, D. S. Stephens, L. Lefkowitz, M. L. Cartter, R. Danila, P. Cieslak, K. A. Shutt, T. Popovic, A. Schuchat, L. H. Harrison, A. L. Reingold, et al. 1999. The changing epidemiology of meningococcal disease in the United States, 1992–1996. J. Infect. Dis. 180:1894–1901.
27. Rosenstein, N. E., B. A. Perkins, D. S. Stephens, T. Popovic, and J. M. Hughes. 2001. Meningococcal disease. N. Engl. J. Med. 344:1378–1388.
28. Tsang, R. S., S. G. Squires, W. D. Zollinger, and F. E. Ashton. 2002. Distribution of serogroups of Neisseria meningitidis and antigenic characterization of serogroup Y meningococci in Canada, January 1, 1999 to June 30, 2001. Can. J. Infect. Dis. 13:391–396.
29. van der Ende, A., I. G. A. Schaumana, C. T. P. Hopman, C. A. P. Fuen, and J. Dankert. 1995. Comparison of commercial diagnostic tests for identification of serogroup antigens of Neisseria meningitidis. J. Clin. Microbiol. 33:3326–3327.
30. Vedros, N. A. 1987. Development of meningococcal serogroups, p. 33–37. In N. A. Vedros (ed.), Evolution of meningococcal disease, vol. II. CRC Press, Boca Raton, Fla.
31. Vogel, U., H. Claus, and M. Frosch. 2001. Capsular operons. Methods Mol. Med. 67:187–201.
32. Zimmerman, S. E., and J. W. Smith. 1978. Identification and grouping of Neisseria meningitidis directly on agar plates by coagglutination with specific antibody-coated protein A-containing staphylococci. J. Clin. Microbiol. 7:470–473.