Direct and Rapid Identification and Genogrouping of Meningococci and *porA* Amplification by LightCycler PCR

Paula Mölling,* Susanne Jacobsson, Anders Bäckman, and Per Olcén

National Reference Laboratory for Pathogenic *Neisseria* at the Department of Clinical Microbiology, Örebro University Hospital, Örebro, Sweden

Received 20 May 2002/Returned for modification 5 August 2002/Accepted 26 September 2002

Invasive meningococcal infections are usually diagnosed by culture. This approach is far from optimal due to, e.g., treatment with precollection antibiotics. Molecular-genetics methods are therefore recognized as important tools for optimal laboratory confirmation of meningococcal infections as well as characterization of meningococci (Mc). The aims of the present study were to develop real-time PCRs for identification and genogrouping (A, B, C, Y, and W-135) of Mc and *porA* amplification that further can be used for subsequent genosubtyping. In a first run Mc were identified. In a second run they were genogrouped and *porA* genes were amplified. All the Mc isolates ($n = 71$) but one and cerebrospinal fluid samples ($n = 11$) tested gave the expected positive results. The specificity, inter- and intra-assay variations, and effects of different amounts of DNA on the melting temperatures were also explored. The LightCycler PCR system was found to effectively detect and characterize Mc in a few hours. This testing, including the DNA sequencing of the *porA* gene to reveal the genosubtype, does not take more than a working day, and the results can be compared to those from culturing with phenotypic analysis, which takes at least a few days.

Neisseria meningitidis is one of the major causes of meningitis and septicemia throughout the world, with high morbidity and mortality. Invasive meningococcal infection can be diagnosed by culture of cerebrospinal fluid (CSF) and blood, which takes 24 h or more. Isolation of meningococci (Mc) also allows antibiotic susceptibility testing and phenotypic characterization. Culturing of Mc is, however, not optimal due to the increased use of precollection antibiotics. Nonculture diagnostic methods have recently been recognized as an increasingly important tool for optimal laboratory confirmation of meningococcal infections. Detection of nucleic acids of the 16S rRNA gene by PCR has been used for diagnosis of meningococcal disease (2, 15, 16, 19, 25). Recently, PCR assays have also focused on the conserved regulatory gene, *crgA* (28), as well as the *ctrA* gene (7, 13, 17, 24), a target involved in the transport of the capsular polysaccharide (10).

Based on chemical differences in the bacterial polysaccharide capsules, at least 13 serogroups have been defined by serological specificity against these capsules (23). Five of the serogroups (A, B, C, Y, and W-135) are responsible for most meningococcal disease, with group A, B, and C bacteria accounting for about 90% of the cases (23). Other important antigens of the Mc are the subcapsular outer membrane proteins, PorA and PorB. Their variations form the basis for strain characterization by serosubtyping and serotyping (9). All the serological methods require a culture-positive sample or detection of the antigen as such. Problems with false-negative cultures are common, especially in developing countries where serogroup A Mc are a major cause of epidemics. Serogroup A Mc express a capsular polysaccharide of nonsialic acid (18).

The biosynthesis of the A capsule is controlled by a gene cassette containing four open reading frames (ORFs), *sacA* to *-D* (27). These ORFs have not been found in the genomes of the other meningococcal groups and consequently enable specific detection of the nucleic acids of this group by PCR (22, 28). ORF 3 (*sacC*) and its product do not have homology with other genes and predicted proteins, respectively, in the computerized nucleotide and amino acid databases, and so ORF 3 was chosen as a target in the present study. Serogroup B, C, Y, and W-135 Mc all contain gene cassettes that control the expression of polysaccharide capsules of different sialic acids (3, 4). These cassettes also contain four ORFs, *siaA* to *-D*, and the *siaD* gene has been used as the target for genogrouping B, C, Y, and W-135 Mc by PCR enzyme-linked immunosorbent assay (5, 6) and PCR (7, 13, 24, 28). Molecular techniques for genosubtyping are based on PCR amplification followed by the sequencing of the variable regions (VRs) within the *porA* gene, encoding PorA (1, 8, 20, 26).

The aim of the present study was to develop real-time PCRs for identification and genogrouping of Mc and *porA* amplification.

MATERIALS AND METHODS

Bacterial strains. A total of 71 different Mc isolates were used. The clinical isolates ($n = 63$) were specimens of CSF or blood in various Swedish and African laboratories and were preserved at -70°C in the Swedish Reference Laboratory for pathogenic *Neisseria*. The isolates were of serogroups A ($n = 11$), B ($n = 11$), C ($n = 11$), Y ($n = 11$), W-135 ($n = 11$), 29-E ($n = 3$), X ($n = 1$), and Z ($n = 1$), and there were three nonserogroupable Mc. The other isolates were reference strains ($n = 8$) of groups A (OR173/87), B (NCTC 10026), C (NCTC 8554), Y (NCTC 10791), W-135 (NCTC 11203), 29-E (NCTC 10793), X (NCTC 10790), and Z (NCTC 10792).

The meningococcal isolates were cultured on GCSP agar (3% GC medium base [Difco Laboratories, Detroit, Mich.], with 0.4% D-glucose, 0.01% L-glutamine, 0.0001% cocarboxylase, 0.0005% ferric nitrate, and 0.5% IsoVitaleX enrichment [BBL, Becton Dickinson Europe]) for 18 to 20 h at 37°C in 5% CO_2 . They were serogrouped by coagglutination (21).

Isolates of other bacterial species were also included: one *Bacillus pumilus*

* Corresponding author. Mailing address: Department of Clinical Microbiology, Örebro University Hospital, SE-701 85 Örebro, Sweden. Phone: 46 19 602 15 20. Fax: 46 19 12 74 16. E-mail: paula.molling@orebroll.se.

TABLE 1. Design of the meningococcal LightCycler PCR

Assay	MgCl ₂ concn (mM)	Primers (concn [μ M]) ^a	PCR program ^b
Mc identification	4	NM (0.3), Mod ru8 (0.5)	I
Genogrouping	3	McA F2 (0.5), McB R2 (0.5)	II
	3	McB F3 (0.3), McB R3 (0.5)	II
	3	McC F3 (0.5), McC R3 (0.5)	II
	3	McY F3 (0.3), McY R1 (0.3)	II
	4	McW-135 F3 (0.5), McW-135 R1 (0.5)	II
<i>porA</i> amplification	4	PorA F1 (0.5), PorA R1 (0.5), PorA F2 (0.3), PorA R2 (0.5)	II

^a See Table 2.^b See Materials and Methods.

(CCUG 3273) isolate and one *Escherichia coli* K51 (CCUG 11300) isolate, both known to have cross-reactivity to the group A meningococcal capsular antigen (12, 29), and an *E. coli* K1 (01K1/78) strain and an *E. coli* K92 (CCUG 11375) strain, known to have cross-reactivity to the group B and C meningococcal capsular antigens, respectively (11, 14). The isolates were cultured on blood agar (4.25% Columbia II agar [BBL], 0.3% agar no. 2 [Lab M, Lancashire, England], and 5% defibrinated horse blood [SVA, Håttunaholm, Sweden]) overnight at 37°C.

In addition, isolates of bacterial species that commonly cause bacterial meningitis were tested. The isolates consisted of the following reference strains and clinical isolates: three *Streptococcus pneumoniae* strains of type 1 (SSI SP 1/1), type 2 (SSI SP 2/2), and type 119 (CCUG 36696); three group B streptococci of type 1c (CCUG 22012), type III (CCUG 29782), and type IV (CCUG 29783); and three *Listeria monocytogenes* isolates (B130/84, P48/88, and P161/91). All these bacteria were cultured on blood agar. Three reference strains of *Haemophilus influenzae* (type b [CCUG 23946], type c [CCUG 4852], and type d [CCUG 18372]) were also included and were cultured overnight on GCAGP agar (3.6% GC II agar base [BBL], 1% hemoglobin powder [BBL], 10% horse serum [SVA], and 1% IsoVitalX enrichment [BBL]) at 37°C with 5% CO₂.

CSF samples. Culture-positive CSF samples ($n = 11$) from various Swedish and African patients with meningococcal meningitis were assayed. The corresponding isolates were of serogroups A ($n = 5$), B ($n = 5$), and C ($n = 1$). The CSFs containing Mc of serogroup A came from Sudan in 1985 and 1988, and the CSFs containing Mc of serogroups B and C were all from Sweden (1988 to 1995).

DNA isolation. The DNA from cultured isolates and CSF samples was prepared by using the Dynabeads DNA DIRECT system I (DynaL, Oslo, Norway)

according to the producer's protocol. Samples of 50 μ l of CSF or 20 μ l of suspended bacteria (a loop of 1 μ l of bacteria suspended in sterile distilled water) were incubated with the lysis buffer with Dynabeads at 65°C for 15 min. The DNA was washed twice and then eluted from the beads during incubation at 65°C for 5 min. The DNA preparations were stored at 4°C prior to PCR.

LightCycler PCR. The identification and genogrouping (A, B, C, Y, and W-135) of Mc and the *porA* amplification were run in seven separate reactions (Table 1). The primers used were designed to fit in either of two different PCR programs, I or II (Table 2). The amplification within the *porA* gene was designed to give two amplicons, one from VR1 and the other from VR2 and VR3. The PCRs were performed in a LightCycler system (Roche Molecular Biochemicals, Mannheim, Germany) using SYBR Green fluorescence melting curve analysis for detection and identification of amplicons. In this system all reactions are run in glass capillaries with a total volume of 20 μ l. The reaction mixture consisted of 2 μ l of FastStart DNA Master SYBR Green I (FastStart Taq DNA polymerase, reaction buffer, deoxynucleoside triphosphate mixture [with dUTP instead of dTTP], SYBR Green I dye, and 10 mM MgCl₂) (Roche Diagnostics GmbH, Mannheim, Germany). Primers were added to a final concentration of 0.3 to 0.5 μ M, and MgCl₂ was added to a final concentration of 3 to 4 mM (Table 1), together with 2 μ l of the extracted DNA (DNA from all bacterial isolates was first diluted 1:10). In each run, one positive control and one negative control were included. The reference strains (20 ng/ml) from groups A, B, C, Y, and W-135 were positive controls in the corresponding genogroup reaction. The group A reference was used as positive control in the identification of Mc as well as in the *porA* amplification. As a negative control, water was added instead of DNA.

The PCR programs start with a preincubation for activation of the FastStart enzyme, continue with amplification, and end with a melting curve analysis. The temperature transition rate was 20°C/s. PCR program I was performed by using the following parameters: preincubation at 95°C for 10 min and amplification with 40 cycles of denaturation at 95°C for 15 s, annealing at 54°C for 5 s, and extension at 72°C for 35 s. Heating the product at 95°C, cooling it at 65°C for 15 s, and then slowly heating the product at 0.1°C/s to 95°C were performed for the melting-curve analysis. Program II was performed with similar parameters (95°C for 10 min and 35 cycles of 95°C for 15 s, 59°C for 5 s, and 72°C for 15 s); the melting-curve analysis consisted of heating at 95°C, cooling at 45°C for 15 s, and heating at 0.1°C/s up to 95°C. Fluorescence was measured at the end of each extension step for real-time visualization. For identification of a specific PCR product, the melting temperature (T_m) of the product was given by the fluorescence, which was continuously measured during the slow heating of the melting curve analysis. However, to improve the visualization of this T_m , melting peaks were derived from the initial melting curves (fluorescence [F_1] versus temperature [T]) by plotting the negative derivative of fluorescence over temperature versus temperature ($-dF_1/dT$ versus T) (Fig. 1).

The mean T_m was calculated by using the specific T_m results from all runs for the isolates tested ($n = 71$) and the CSF samples ($n = 11$). Serial dilutions of 2 μ g/ml to 2 pg/ml of bacterial DNA (conversion factor: 4 fg of DNA $\approx$ 1 bacterial

TABLE 2. Primers used in the present study

Primer(s) ^a	Sequence 5'-3'	Position (bp)	Target gene	Accession no. ^c
NM ^b	TGTTGGGCAACCTGATTG	803-820	16S rRNA	AF059671
Mod ru8 ^b	TGATCCA(G/A)CCGCA(G/C)(G/C)TTC	Universal		
McA F2	AAGATGATGCTAGAGGCACT	3523-3542	<i>sacC</i>	AF019760
McA R2	GAATGGTTTTTCGAGCGTGT	3765-3747		
McB F3	ACACCATTACTCTCACCTCAAC	4130-4152	<i>siaD</i>	M95053
McB R3	CTTGGATCATTTTCAGTTTTCAC	4232-4208		
McC F3	TTGGACTGACATCGACTTCTATTGTT	971-996	<i>siaD</i> (<i>synE</i>)	U75650
McC R3	GGTGTCTCTTGTGGGCTGTAT	1089-1067		
McY F3 and W-135 F3	AAGGTGAATCTTCCGAGCAGGA	1005-1026	<i>siaD</i>	Y13969, Y13970
McY R1	GATATCGTACACCATACCTCTAGA	1114-1090		Y13969
McW R1	TTAAAAACACAACCATTTTCATTATAGTTACTGTA	1188-1154		Y13970
PorA F1	GGCGAAATCAAAGCCGGCGT	91-110	<i>porA</i>	AF239810
PorA R1	TAAAGCCGATAAAGGAGCCGAAATC	235-211		
PorA F2	CGGCACGT(C/T)CAATTCTTC	531-550		
PorA R2	CAAGCCGCCTTCCTCATAGC	858-839		

^a Synthesized by Scandinavian Gene Synthesis AB, Köping, Sweden.^b The primers were the same as those used by Bäckman et al. (2), with the exception that ru8 was shortened.^c Accession numbers for nucleotide sequences registered in the databases available at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>).

TABLE 3. Positive results and T_m analyses from all meningococcal reference and clinical isolates ($n = 71$) and CSFs ($n = 11$)

Assay	Sample	No. positive/ no. tested ^c	Mean T_m (°C) (SD)	Range (°C)
Mc identification 16S rRNA	Isolate ^a	71/71	89.0 (0.2)	88.6–89.4
	CSF	11/11	89.7 (0.1)	89.5–89.9
Mc A grouping	Isolate	12/12	79.8 (0.1)	79.7–80.3
	CSF	5/5	80.0 (0.3)	79.5–80.3
Mc B grouping	Isolate	12/12	76.9 (0.1)	76.7–77.0
	Ng ^{a,d}	1	77.5	
Mc C grouping	CSF	5/5	76.6 (0.2)	76.4–76.9
	Isolate	12/12	81.4 (0.1)	81.2–81.6
Mc Y grouping	Ng ^a	2	81.8 (0.007)	81.8
	CSF	1/1	81.9	
Mc W-135 grouping	Isolate	12/12	78.0 (0.1)	77.8–78.1
<i>porA</i> amplification	Isolate ^a	70 ^b /71	87.4 (0.5)	86.4–88.3
	CSF	11/11	87.4 (0.4)	86.7–87.8

^a Samples were run at different times, therefore interassay variations are included; see Table 4.

^b The group B reference strain showed only two weak peaks. Two of the group C isolates also showed an additional peak at a T_m of 90.3°C.

^c For the nonserogroupable isolates, the number is the number of such isolates identified.

^d Ng, nonserogroupable isolate.

genome) from the meningococcal reference strains of groups A, B, C, Y, and W-135 were run to test the detection level as well as the effect on T_m of different target DNA concentrations. Interassay variation was tested by using 20 ng of the reference strains/ml during four or five different runs. Intra-assay variation was tested with one of the reference strains (20 ng/ml) in different capillaries in the same run.

Confirmation of the LightCycler PCR amplicons. Gel electrophoresis and DNA sequencing initially verified the meningococcal amplicons from the different protocols. The *porA* amplicons from all reference strains ($n = 8$) were also sequenced to reveal the genosubtype as described before (20). The electrophoresis was run on a 1% agarose gel for the 16S rDNA product and on a 2% agarose gel for the capsules and the *porA* products with ethidium bromide.

The LightCycler amplicons were purified by the High Pure PCR product purification kit (Boehringer Mannheim, Indianapolis, Ind.) and then cycle sequenced with the same primers that made each amplicon (Table 2) by using the BigDye terminator cycle sequencing kit (Applied Biosystems, Warrington, England). Completed reaction mixtures were subjected to capillary electrophoresis using an ABI PRISM 310 genetic analyzer (Applied Biosystems). The results were then compared with nucleotide sequences registered in the databases available at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) by BLAST similarity search.

RESULTS

All reference strains ($n = 8$) were positive for Mc by using the 16S rRNA gene. Groups A, B, C, Y, and W-135 were positive with the corresponding genogroup protocol and negative with the others. Groups 29-E, X, and Z were negative with all the genogroup protocols. Well-defined peaks were observed for all but the group B reference strain, and *porA* amplicons were thereby identified (Table 3). For all strains except the group B strain, which showed a mixture of DNA sequences in each amplicon, the genosubtype could be revealed.

All the clinical meningococcal isolates tested ($n = 63$) were positive for 16S rDNA. Serogroup A, B, C, Y, and W-135 Mc, together with the three nonserogroupable strains, one of which was genogrouped as B and two of which were genogrouped as

C, were all positive with the individual genogroup protocol. In all strains *porA* amplification was also observed, as shown by a well-defined peak together with an additional peak in two of the group C isolates (Table 3).

When other bacterial isolates (known to be cross-reactive with meningococcal capsular antigens or common causes of bacterial meningitis) in the meningococcal identification assay (I) were tested, only the *E. coli* strains gave products with T_m s close to that of products of Mc but 0.5 to 0.7°C lower than that of products of the positive control in each run. In the initial verification of the LightCycler amplicons by gel electrophoresis the *E. coli* products were distinguished from the Mc product by their sizes, which were around 200 bp compared to 710 bp for the Mc amplicons. In the genogrouping none of these isolates gave any “false-positive” results. However, in the *porA* amplification the *B. pumilus* strain gave a product with a T_m similar to, but 0.4°C lower than, that for the product of the positive control.

All the 11 CSF samples were positive for meningococcal 16S rDNA, as well as with the individual genogroup protocol and in the *porA* amplification (Table 3).

Detection levels. The detection limits of the PCRs were found to be one bacterial genome per PCR (2 pg/ml) in the assay for identification of Mc, 10 copies (20 pg/ml) in genogrouping, and 100 copies (200 pg/ml) in the *porA* gene amplification. For one of these runs using the 16S rDNA as the target for identification of Mc, the melting peaks are shown in Fig. 1. There was a tendency in all the runs for less DNA to give a somewhat higher T_m (Fig. 1). All reactions involving between 20 and 0.2 ng of DNA showed a mean T_m difference of 0.4°C (standard deviation, 0.2°C).

Variation of the assay. The interassay variations, tested by using the same amount of reference DNA from each group (20 ng/ml) in four or five different runs, are shown in Table 4. The intra-assay test, using the Mc W-135 reference strain (20 pg of DNA/ml) in five different capillaries in the same run, showed a T_m range of 78.1 to 78.2°C (Table 4).

Mc amplicon confirmation. The DNA sequencing combined with BLAST similarity search confirmed that the right fragment within each gene had been correctly amplified.

DISCUSSION

The LightCycler system was shown to be an effective system because it was fast and gave correct identification and characterization of Mc. In the first run Mc were identified, and in the second run they were genogrouped (A, B, C, Y, and W-135), together with amplification of the *porA* gene (Table 3). The DNA of the *porA* amplicons can be further sequenced, as shown for all but one of the reference strains, for genosubtyping of Mc (20). The PCRs showed detection limits of 1, 10, and 100 bacterial genomes per PCR for identification of Mc, genogrouping, and *porA* amplification, respectively.

It is difficult to calculate a general “identification T_m ” of a product, since fairly high interassay variations were found. Differences of more than 1°C in the specific T_m between different runs using the same DNA sample could be noticed (Table 4). Different amounts of DNA also had an effect on the T_m (Fig. 1). In contrast, the intra-assay variations were small (<0.1°C), showing that the LightCycler supplies consistent

FIG. 1. Melting peaks from different serial dilutions of the group A reference strain in the PCR for identification of Mc using the 16S rRNA gene. The T_m s of the product were given by the fluorescence continuously measured during a slow-heating melting curve analysis. To improve the visualization of this T_m , the melting peaks were derived from the initial melting curves (fluorescence [F_1] versus temperature [T]) by plotting the negative derivative of fluorescence with respect to temperature versus temperature ($-dF_1/dT$ versus T). Samples 1 to 7 consist of different amounts of meningococcal group A DNA, 1 million copies to 1 copy of the bacterial genome per PCR. Sample 8 is the negative control and does not contain any target DNA.

conditions for the different reaction capillaries in the same run (Table 4). These differences were also seen with the CSF samples, which generally gave a higher melting point and a broader range (Table 3) than the isolates, probably because less DNA and salts remained after the DNA isolation. These findings constitute the background for why a standard of known DNA concentration is necessary and must always be included in each run.

In most cases the nonspecific amplicons of the *E. coli* strains in the identification assay for Mc (shown by T_m s 0.5 to 0.7°C lower than those for the Mc) can be distinguished from the specific Mc amplicons by using the identification assay on the 16S rDNA gene. This can further be verified by a positive result in the genogrouping since there was no false-positive reaction with the *E. coli* strains in these reactions. If a sample is negative in the genogrouping, one can always use gel electrophoresis to exclude any possibility of nonspecific reaction with *E. coli*. To get a higher specificity, one can also develop the protocol further to use hybridization probes, a concept that has been used for genogrouping Mc (13).

One melting peak instead of two peaks arises from the *porA* amplicons in most of the isolates but not two group C isolates. Getting two different melting peaks could actually be expected,

TABLE 4. Effect on T_m when using different target DNA concentrations, as well as inter- and intra-assay variations

Assay	DNA usage ^a	No. of samples	Mean T_m (°C) (SD)	Range (°C)
Mc identification	Dilution	7	90.0 (0.1)	89.9–90.2
	Inter-	5	89.3 (0.4)	89.0–90.0
Mc A grouping	Dilution	6	81.5 (0.2)	81.2–81.7
	Inter-	5	80.4 (0.5)	80.0–81.3
Mc B grouping	Dilution	4	78.0 (0.5)	77.6–78.4
	Inter-	5	76.8 (0.5)	76.2–77.6
Mc C grouping	Dilution	4	82.3 (0.4)	82.1–82.9
	Inter-	4	81.6 (0.5)	80.9–82.1
Mc Y grouping	Dilution	6	79.4 (0.5)	78.9–80.3
	Inter-	4	78.3 (0.6)	77.8–79.3
Mc W-135 grouping	Dilution	6	79.3 (0.5)	78.8–79.9
	Inter-	4	78.4 (0.4)	78.2–78.9
	Intra-	5	78.2 (0.03)	78.1–78.2
<i>porA</i> amplification	Dilution	5	88.3 (0.09)	88.3–88.5
	Inter-	5	87.4 (0.4)	87.0–88.1

^a Dilution, different amounts of DNA (between 2 µg/ml and 2 pg/ml) diluted 1:10, 1:100, and so on, for each sample used in the same run; inter-, the same amount of reference DNA from each group (20 ng/ml) used in four or five different runs; intra-, the Mc W-135 reference (20 pg/ml) used in five different capillaries during one run.

since the reaction is designed to give two amplicons of different sizes, one for VR1 and the other for VR2 and VR3, which all are different in both length and G+C content. However, the high G+C contents in most of these amplicons give similar T_m s for their melting peaks, which sometimes can be indistinguishable. This makes it difficult to use only *porA* gene amplification for identification of all Mc when SYBR Green is used as the detection system, but, as a template for further sequencing for genosubtyping, *porA* gene amplification works well. This concept is used routinely in our laboratory, where all Mc are genosubtyped by this method. The group B reference strain that failed to be genosubtyped, due to mixed sequences, shows unspecific amplification and that some internal sequencing primers may be better than the PCR primers for DNA sequencing. The *porA* gene in this strain must, however, be further investigated, since it has been shown to be nonsero-subtypeable.

In conclusion, real-time PCR makes it possible to detect and characterize Mc in a few hours. This concept, including the DNA sequencing of the *porA* gene to reveal the genosubtype, does not take more than a working day. This can be compared to culturing with subsequent analysis, which takes at least a few days and which does not always give optimal results.

ACKNOWLEDGMENTS

This study was supported by grants from the Örebro County Council Research Committee and the Foundation for Medical Research at Örebro Medical Center Hospital, Sweden.

REFERENCES

- Arhin, F. F., F. Moreau, J. W. Coulton, and E. L. Mills. 1997. Subtyping of *Neisseria meningitidis* strains isolated in Quebec, Canada: correlation between deduced amino acid sequences and serosubtyping techniques. *Can. J. Microbiol.* **43**:234–238.
- Bäckman, A., P. Lantz, P. Rådstrom, and P. Olcén. 1999. Evaluation of an extended diagnostic PCR assay for detection and verification of the common causes of bacterial meningitis in CSF and other biological samples. *Mol. Cell. Probes* **13**:49–60.
- Bhattacharjee, A. K., H. J. Jennings, C. P. Kenny, A. Martin, and I. C. Smith. 1975. Structural determination of the sialic acid polysaccharide antigens of *Neisseria meningitidis* serogroups B and C with carbon 13 nuclear magnetic resonance. *J. Biol. Chem.* **250**:1926–1932.
- Bhattacharjee, A. K., H. J. Jennings, C. P. Kenny, A. Martin, and I. C. Smith. 1976. Structural determination of the polysaccharide antigens of *Neisseria meningitidis* serogroups Y, W-135, and BO1. *Can. J. Biochem.* **54**:1–8.
- Borrow, R., H. Claus, U. Chaudhry, M. Guiver, E. B. Kaczmarek, M. Frosch, and A. J. Fox. 1998. *siaD* PCR ELISA for confirmation and identification of serogroup Y and W135 meningococcal infections. *FEMS Microbiol. Lett.* **159**:209–214.
- Borrow, R., H. Claus, M. Guiver, L. Smart, D. M. Jones, E. B. Kaczmarek, M. Frosch, and A. J. Fox. 1997. Non-culture diagnosis and serogroup determination of meningococcal B and C infection by a sialyltransferase (*siaD*) PCR ELISA. *Epidemiol. Infect.* **118**:111–117.
- Corless, C. E., M. Guiver, R. Borrow, V. Edwards-Jones, A. J. Fox, and E. B. Kaczmarek. 2001. Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. *J. Clin. Microbiol.* **39**:1553–1558.
- Feavers, I. M., A. J. Fox, S. Gray, D. M. Jones, and M. C. Maiden. 1996. Antigenic diversity of meningococcal outer membrane protein PorA has implications for epidemiological analysis and vaccine design. *Clin. Diagn. Lab. Immunol.* **3**:444–450.
- Frasch, C. E., W. D. Zollinger, and J. T. Poolman. 1985. Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes. *Rev. Infect. Dis.* **7**:504–510.
- Frosch, M., D. Muller, K. Bousset, and A. Muller. 1992. Conserved outer membrane protein of *Neisseria meningitidis* involved in capsule expression. *Infect. Immun.* **60**:798–803.
- Glode, M. P., J. B. Robbins, T. Y. Liu, E. C. Gotschlich, I. Orskov, and F. Orskov. 1977. Cross-antigenicity and immunogenicity between capsular polysaccharides of group C *Neisseria meningitidis* and of *Escherichia coli* K92. *J. Infect. Dis.* **135**:94–104.
- Guirguis, N., R. Schneerson, A. Bax, W. Egan, J. B. Robbins, J. Shiloach, I. Orskov, F. Orskov, and A. el Kholy. 1985. *Escherichia coli* K51 and K93 capsular polysaccharides are crossreactive with the group A capsular polysaccharide of *Neisseria meningitidis*. Immunochemical, biological, and epidemiological studies. *J. Exp. Med.* **162**:1837–1851.
- Guiver, M., R. Borrow, J. Marsh, S. J. Gray, E. B. Kaczmarek, D. Howells, P. Boseley, and A. J. Fox. 2000. Evaluation of the Applied Biosystems automated Taqman polymerase chain reaction system for the detection of meningococcal DNA. *FEMS Immunol. Med. Microbiol.* **28**:173–179.
- Kasper, D. L., J. L. Winkelhake, W. D. Zollinger, B. L. Brandt, and M. S. Artenstein. 1973. Immunochemical similarity between polysaccharide antigens of *Escherichia coli* 07: K1(L)NM and group B *Neisseria meningitidis*. *J. Immunol.* **110**:262–268.
- Kotilainen, P., J. Jalava, O. Meurman, O. P. Lehtonen, E. Rintala, O. P. Seppala, E. Eerola, and S. Nikkari. 1998. Diagnosis of meningococcal meningitis by broad-range bacterial PCR with cerebrospinal fluid. *J. Clin. Microbiol.* **36**:2205–2209.
- Kristiansen, B. E., E. Ask, A. Jenkins, C. Fermer, P. Rådstrom, and O. Sköld. 1991. Rapid diagnosis of meningococcal meningitis by polymerase chain reaction. *Lancet* **337**:1568–1569.
- Lansac, N., F. J. Picard, C. Menard, M. Boissinot, M. Ouellette, P. H. Roy, and M. G. Bergeron. 2000. Novel genus-specific PCR-based assays for rapid identification of *Neisseria* species and *Neisseria meningitidis*. *Eur. J. Clin. Microbiol. Infect. Dis.* **19**:443–451.
- Liu, T. Y., E. C. Gotschlich, F. T. Dunne, and E. K. Jonssen. 1971. Studies on the meningococcal polysaccharides. II. Composition and chemical properties of the group B and group C polysaccharide. *J. Biol. Chem.* **246**:4703–4712.
- McLaughlin, G. L., D. K. Howe, D. R. Biggs, A. R. Smith, P. Ludwinski, B. C. Fox, D. N. Tripathy, C. E. Frasch, J. D. Wenger, R. B. Carey, et al. 1993. Amplification of rDNA loci to detect and type *Neisseria meningitidis* and other eubacteria. *Mol. Cell. Probes* **7**:7–17.
- Mölling, P., M. Unemo, A. Bäckman, and P. Olcén. 2000. Genosubtyping by sequencing group A, B and C meningococci; a tool for epidemiological studies of epidemics, clusters and sporadic cases. *APMIS* **108**:509–516.
- Olcén, P., D. Danielsson, and J. Kjellander. 1975. The use of protein A-containing staphylococci sensitized with anti-meningococcal antibodies for grouping *Neisseria meningitidis* and demonstration of meningococcal antigen in cerebrospinal fluid. *Acta Pathol. Microbiol. Scand.* **83**:387–396.
- Orvelid, P., A. Bäckman, and P. Olcén. 1999. PCR identification of the group A *Neisseria meningitidis* gene in cerebrospinal fluid. *Scand. J. Infect. Dis.* **31**:481–483.
- Peltola, H. 1983. Meningococcal disease: still with us. *Rev. Infect. Dis.* **5**:71–91.
- Porritt, R. J., J. L. Mercer, and R. Munro. 2000. Detection and serogroup determination of *Neisseria meningitidis* in CSF by polymerase chain reaction (PCR). *Pathology* **32**:42–45.
- Rådstrom, P., A. Bäckman, N. Qian, P. Kragstjerg, C. Pålsson, and P. Olcén. 1994. Detection of bacterial DNA in cerebrospinal fluid by an assay for simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and streptococci using a seminested PCR strategy. *J. Clin. Microbiol.* **32**:2738–2744.
- Swaminathan, B., G. M. Matar, M. W. Reeves, L. M. Graves, G. Ajello, W. F. Bibb, L. O. Helsel, M. Morales, H. Dronavalli, M. el-Swify, W. DeWitt, and S. B. Hunter. 1996. Molecular subtyping of *Neisseria meningitidis* serogroup B: comparison of five methods. *J. Clin. Microbiol.* **34**:1468–1473.
- Swartley, J. S., L.-J. Liu, Y. K. Miller, L. E. Martin, S. Edupuganti, and D. S. Stephens. 1998. Characterization of the gene cassette required for biosynthesis of the (α 1 $\rightarrow$ 6)-linked *N*-acetyl-D-mannosamine-1-phosphate capsule of serogroup A *Neisseria meningitidis*. *J. Bacteriol.* **180**:1533–1539.
- Taha, M. K. 2000. Simultaneous approach for nonculture PCR-based identification and serogroup prediction of *Neisseria meningitidis*. *J. Clin. Microbiol.* **38**:855–857.
- Vann, W. F., T. Y. Liu, and J. B. Robbins. 1976. *Bacillus pumilus* polysaccharide cross-reactive with meningococcal group A polysaccharide. *Infect. Immun.* **13**:1654–1662.