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# PCR-Based Assay for Detection of *Neisseria meningitidis* Capsular Serogroups 29E, X, and Z

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**PCR-based assays for the identification of *Neisseria meningitidis* serogroups 29E, X, and Z by detection of specific regions of the *ctrA* gene are described. The specificities of these assays were confirmed using serogroups A, B, C, 29E, H, W135, X, Y, and Z and nongroupable meningococcal isolates.**

Characterization of meningococci by serogroup is useful and convenient in investigation of disease outbreaks and vaccine effectiveness. Thirteen meningococcal serogroups are known based on capsular polysaccharide antigens of viable organisms (17, 21). PCR-based assays are routinely used for the detection and identification of serogroups A, B, C, W135, and Y (2, 4, 5, 8, 9, 12, 15, 16, 22, 25; M.-K. Taha and A. Fox, 7th Meet. Eur. Monit. Group Meningococci, abstr. P39-40, 2003). In Ireland, serogroup B, C, W135, and Y meningococci were responsible for 97% of cases diagnosed in 1997 to 2000, with group B predominating (M. Cafferkey, K. Murphy, M. Fitzgerald, and D. O'Flanagan, 6th Meet. Eur. Monit. Group Meningococci, abstr. P67-68, 2001), similar to the situations in other developed countries (17, 19); hence, only 3% of cases were caused by other serogroups. PCR-based assays are not available for the identification of serogroups 29E, X, and Z. It is possible that isolates designated as nongroupable are isolates of these serogroups that fail to express or poorly express the capsular phenotype while harboring the genetic material necessary for capsule production.

A limited number of cases of meningococcal disease (1%) due to serogroups 29E, X, and Z have been reported worldwide (7, 14, 20, 22). This low incidence is probably an underestimation. Strains of these three serogroups have been isolated in carriage studies, albeit also at low frequencies, with reported figures of 5.6 and 5.8% for serogroup 29E (1, 10), 2.1 and 3.8% for serogroup X (1, 10), and from 0.6 to 6.6% for serogroup Z (1, 3), with a median of 1.3% (7, 10, 13).

Here we report PCR-based assays for the rapid individual detection and identification of meningococcal serogroups 29E, X, and Z. The *ctrA* gene, exclusive to meningococci and forming part of the capsule biosynthesis locus, was chosen as the PCR target. The 3′ end of the *ctrA* gene is highly conserved among meningococci irrespective of serogroup, but the 5′ end varies. It is to this variable region that forward primers specific for serogroups 29E, X, and Z (Table 1) were designed (GenBank accession numbers AF520907, AY289931, and AF520909, respectively). These primers were used with a reverse primer (Table 1) described by Corless et al. (4) and demonstrated to amplify a region of the *ctrA* gene from all meningococcal serogroups (4, 11).

The specificity of these primers in amplifying the *ctrA* target from different meningococcal serogroups was tested by PCR. All meningococcal strains used were isolated from cases of disease or during a carriage survey (unpublished data). The serogroup of each isolate was determined by coagglutination and/or by direct slide agglutination. For each serogroup, separate PCRs were performed in 50-μl volumes comprising 1 U of *Taq* DNA polymerase (Gibco-BRL/Life Technologies), 2.5 mM $MgCl_2$, 5 μl of 10× buffer (1× buffer contains 10 mM Tris HCl [pH 9 at 25°C], 50 mM KCl, 0.1% Triton X-100), 1.5 mM (each) forward and reverse primer, and 0.2 mM deoxynucleoside triphosphates (dATP, dGTP, dCTP, and dTTP), to which 1 μl of purified *N. meningitidis* DNA (100 to 300 ng) (Puregene DNA isolation kit; Gentra Systems, Minneapolis, Minn.) was added. Amplifications were carried out on a PTC 200 DNA Engine (MJ Research) thermal cycler, using the same parameters irrespective of serogroup, and involved, after an initial cycle of 95°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 62.5°C for 1 min, and extension at 72°C for 30 s. Following amplification, all reaction mixes were held at 4°C until 5 μl of reaction mix was analyzed on a 3.5% (wt/vol) agarose gel containing 0.5 μg of ethidium bromide/ml and viewed using UV light.

Amplification products of 667 bp were observed for serogroups 29E (primer *ctrA* 29EF2) and Z (primer *ctrA* ZF), and a product of 525 bp was observed for serogroup X (primer *ctrA* XF3). No cross-reactions were observed between the specific forward primer and isolates of either of the other serogroups or for representative strains of serogroups B, C, H, W135, and Y (including 10 serogroup B strains, 10 serogroup C strains, 2 serogroup H strains, 5 serogroup W135 strains, and 5 serogroup Y strains). With the *ctrA* XF3 primer, products were detected in two serogroup A strains but were of a different size (approximately 650 bp) from that observed with serogroup X strains. Compared to conventional serologic grouping, the assays correctly identified 12 of 12 serogroup X isolates, 4 of 4 serogroup Z isolates, and 2 of 2 serogroup 29E isolates. Therefore, the specificity of these primers for grouping strains of these serogroups was 100%.

To further evaluate these assays, we applied them to 37

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TABLE 1. Primer sequences used in this study

| Name | 5′–3′ sequence | Reference |
|---|---|---|
| *ctrA* 29EF2 | ATTACGCTGACGGCATGTGGA | This study |
| *ctrA* ZF | TATGCGGTGCTGTTCGCTATG | This study |
| *ctrA* XF3 | GTCTTTGTATAAGGCCCAAG | This study |
| *ctrA* UR | TTGTCGCGGATTTGCAACTA | 5 |

isolates for which the serogroups could not be identified by conventional methods (hence, not of serogroup A, B, C, H, W135, or Y) that were recovered as part of a carriage survey undertaken during the 2000-2001 epidemiological year (D. E. Bennett, A. D. Stack, and M. T. Cafferkey, unpublished data). Each of these isolates contained the *ctrA* gene. Of the 37, 11 yielded a product with the *ctrA* XF3/*ctrA* UR primers, 6 yielded a product with the *ctrA* ZF/*ctrA* UR primers, and 19 yielded a product with the *ctrA* 29EF2/*ctrA* UR primers. The three primer pairs failed to generate an amplification product with only 1 isolate; presumably this isolate represented another uncommon serogroup, for instance, serogroup I, K, or L (for which there are no antisera available).

This report demonstrates that these assays reliably distinguish meningococcal serogroups 29E, X, and Z from other serogroups, with the major benefit of identifying these among previously nongroupable isolates. Used in conjunction with the already available PCR-based identification assays (6, 8, 15), these new assays allow the characterization of all meningococcal serogroups currently known to be associated with disease and/or carriage worldwide. Furthermore, since these assays are PCR based, they could be applied to clinical samples for diagnostic purposes.

Although few cases of disease have been reported with these serogroups, temporal changes in disease-associated serogroups are well documented (23); hence, they may emerge as significant pathogens. Characterization of meningococci according to serogroup is important given that most available meningococcal vaccines are capsular polysaccharide based (18). The exact effects of widespread immunization as far as inducing changes in the population structure of meningococci are unknown, but it is likely to affect the phenomenon of capsule switching and also to select for strains of serogroups previously considered to be of limited pathogenicity, such as serogroups 29E, X, and Z (20, 24). It is probable that the observed replacement of serogroup A strains by serogroup X strains colonizing and causing disease in northern Ghana was a direct effect of the mass A/C polysaccharide vaccination campaigns of 1997 and 1998 (7). The emergent serogroup X strains were closely related to serogroup X strains already present but at a much lower abundance. For precisely this reason, the serogroups of all circulating meningococci should be identified and monitored, especially in countries in which widespread meningococcal vaccination has been introduced.

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