siaD PCR ELISA for confirmation and identification of serogroup Y and W135 meningococcal infections

Ray Borrow a,*, Heike Claus b, Usha Chaudhry a, Malcolm Guiver a, Edward B. Kaczmarski a, Matthias Frosch b, Andrew J. Fox a

a Manchester Public Health Laboratory, Withington Hospital, Nell Lane, West Didsbury, Manchester, M20 2LR, UK
b Institut für Hygiene und Mikrobiologie, Josef-Schneider-Str. 2, D-97080, Würzburg, Germany

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Abstract

Non-culture diagnosis and serogroup determination of meningococcal infection is important in contact management where vaccination may be possible. A serogroup B and C PCR ELISA assay for the non-culture diagnosis and serogroup determination has proved invaluable for enhanced epidemiological surveillance and contact management. A polymerase chain reaction assay, based on a restriction fragment length polymorphism in the meningococcal serogroup Y and W135 sialyltransferase (siaD) gene, was developed to enhance the range of non-culture diagnosis of meningococcal infection from clinical samples. The PCR assay was adapted to an ELISA format incorporating hybridisation with serogroup-specific Y and W135 oligonucleotide probes. The serogroup-specific W135 and Y PCR ELISA is a useful addition to currently available serogroup B and C assay for non-culture diagnosis of meningococcal infection and outbreak investigation. © 1998 Federation of European Microbiological Societies. Published by Elsevier Science B.V.

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1. Introduction

Non-culture diagnosis is being recognised as an increasingly important tool for maximum laboratory confirmation of meningococcal infection. Enhanced surveillance for meningococcal infection to determine the true incidence of infection, is vital to accurately ascertain efficacy of novel polysaccharide-protein conjugate vaccines for serogroup C. These have been shown to be safe and immunogenic in infants and are likely to become available in the near future [1]. Despite the current low incidence of infections of serogroups Y and W135, accurate ascertainment and surveillance for the less common serogroups is important.

In England and Wales in 1995 serogroups B and C accounted for 95.2% of meningococcal infections while 3.5% of infections were due to Y and W135 [2]. However, during 1989 to 1995, active laboratory-based surveillance conducted in a number of counties in the United States showed the proportion of serogroup Y meningococcal disease increased dramat-
ically from 0 in 1989 to 32.5% in 1995 at a time when the overall rate of meningococcal disease remained stable [3].

A number of polymerase chain reaction (PCR) assays for different meningococcal target genes exist and PCR is now used extensively in the non-culture diagnosis of meningococcal infection [4–9]. The $\text{siaD}$ PCR assay described by Borrow et al. (1997) identifies serogroup B and C meningococci [9] which are of greatest value in contact management where vaccination is available for serogroup C disease contacts. A quadrivalent vaccine is available for serogroups A, C, Y and W135 with vaccination recommended for contacts of an index case of any of these serogroups [10]. Using the serogroup-specific sialyltransferase ($\text{siaD}$) genes, sequence differences between serogroups B and C were exploited to enable development of a PCR ELISA assay for the differentiation of serogroups B and C. A similar approach was adopted for serogroups Y and W135, the capsules of which are again biochemically very similar. The serogroup Y polysaccharide is composed of equimolar proportions of $\text{N}$-acetylneuraminic acid and $\text{D}$-glucose and is partially $\text{O}$-acetylated whilst the serogroup W135 polysaccharide capsule only differs by the absence of $\text{O}$-acytelyation and contains $\text{D}$-galactose instead of $\text{D}$-glucose [11]. The DNA sequences of the $\text{siaD}$ genes of serogroups W135 and Y are highly homologous but differ completely from those of serogroups B and C which are distinct but genetically related [12]. From sequence data of the Y and W135 $\text{siaD}$ genes [12] it was however possible to identify a $\text{siaD}$ gene restriction fragment length polymorphism (RFLP) which appeared stable among serogroup Y and W135 meningococci and developed a serogroup-specific Y and W135 PCR assay for the non-culture diagnosis of meningococcal infection in a microtitre colorimetric hybridisation assay (PCR ELISA) format. The initial findings include an evaluation of a $\text{siaD}$ PCR ELISA for the direct non-culture detection and determination of the serogroups Y and W135 by examination of meningococcal DNA in CSF, serum and plasma and improved epidemiological characterisation of non-groupable meningococci.

2. Materials and methods

2.1. Bacterial strains and culture methods

Bacterial strains used were isolates of Neisseria meningitidis referred to the PHLS MRU (Meningococcal Reference Unit, Manchester Public Health Laboratory, Withington Hospital, Manchester, UK). Organisms were cultured on blood agar overnight at 37°C (in an atmosphere of 5% CO$_2$). Freshly cultured organisms were suspended in sterile water and the suspension adjusted to an A$_{650}$ equal to an O.D. of 0.1 and diluted 1/1000 to yield approximately 100 cells/5 μl reaction [9].

Serogrouping of N. meningitidis isolates was performed by co-agglutination using rabbit polyclonal serogroup-specific antisera as previously described [13].

2.2. PCR primers and probes

Primers and probes (synthesised by Oswel DNA Services, University of Southampton, UK) were designed (Primerselect package, DNASTAR Inc., Madison, USA) based upon the serogroup Y and W135 $\text{siaD}$ nucleotide sequences [12].

The serogroup Y and W135 $\text{siaD}$ forward and reverse primers were as follows: 5’ CAAACGGTATCTGATGAAATGCTGGAAG 3’ and 5’ TTA-AAGCTGCGGAAGAATAGTGAAAT 3’. The internal probes specific for serogroup Y and W135 $\text{siaD}$ nucleotide sequences were serogroup Y probe: 5’ CTAATCAGACATCTCAAAGCGAAAGC 3’ and serogroup W135 probe: 5’ TGATCATGACATGAAAGTGAGGGATT 3’.

2.3. DNA amplification

All PCR amplifications were performed as for the serogroup B and C $\text{siaD}$ PCR ELISA [9] with the exception of the annealing step which was performed at 58°C. An aliquot (10 μl) of the amplified product was then electrophoresed on a 2% agarose gel and visualised by ethidium bromide (EtBr) staining. Amplicons were sized against a 100-bp ladder (Pharmacia Biotech, St. Albans, UK) and a positive result recorded if a 530-bp product was present. Negative
(water) and reagent blank controls were included in every set of PCR reactions.

### 2.4. Identification of a RFLP in the siaD gene

To 15 μl of siaD PCR product, 1 μl of XbaI (Northumbria Biologicals Ltd. (NBL), Northumberland, UK), 3 μl of 10X restriction digest buffer (NBL) and 11 μl of deionised, double distilled water were added and the reaction digest was incubated overnight at 37°C. Digests were then analysed by agarose gel electrophoresis and EtBr staining. Fragments were sized by comparison with a 100-bp ladder.

### 2.5. Solution hybridisation by PCR ELISA

The amplification reaction mixture was prepared as for the serogroup B and C siaD PCR ELISA [9] except that the Y and W135 primers were substituted for the degenerate serogroup B and C primers.

Digoxigenin-labelled PCR products were detected according to the manufacturer’s protocol (Boehringer Mannheim, Lewes, UK). Briefly, an aliquot of PCR product was denatured and hybridised separately with serogroup Y or W135 biotinylated capture probes (50 ng/ml of each probe) to allow the immobilisation of the probe to a streptavidin-coated microtitre plate. The bound hybrid was detected by an anti-digoxigenin peroxidase conjugate followed by the addition of the colorimetric substrate ABTS (2,2 azino-di-(3-ethylbenzthiazoline-sulphonate).

### 2.6. Treatment of CSF, plasma and serum samples

CSF samples were collected from patients with culture proven and suspected meningococcal meningitis and stored at −20°C. Aliquots (50 μl) were boiled for 15 min, chilled and centrifuged for 5 min at 14,000×g. An aliquot (5 μl) of treated serum was then used directly in a 50 μl PCR reaction.

### 2.7. Specificities of PCR ELISA using meningococcal cultures

To determine the PCR ELISA specificity, amplification reactions were performed using boiled suspensions of organisms for each of the following meningococcal serogroups (B (n=9), C (n=9), X (n=6), W135 (n=17), Z/29E (n=4), Y (n=15), H (n=3) and A (n=5)).

### 2.8. Evaluation of the siaD PCR ELISA for non-culture serogroup determination of Y and W135 meningococci

The siaD PCR ELISA was evaluated for detection and serogroup identification of serogroups Y and W135 directly from 83 clinical samples (31 CSFs, 13 sera and 39 plasma) received at the MRU from patients with proven or suspected meningococcal infection. These samples had previously tested positive for the presence of meningococcal DNA using a screening PCR ELISA [7] but were negative for serogroups B and C by the serogroup B and C siaD PCR assay [9].

### 3. Results

#### 3.1. Amplification of siaD gene fragments from serogroup Y and W135 meningococci

Amplification of meningococcal DNA from organisms of different serogroups, using the serogroup Y and W135 siaD primers, gave products of the expected size (530 bp) when visualised by EtBr agarose gel electrophoresis for all isolates of serogroup Y and W135 meningococci only (Fig. 1).

#### 3.2. Differentiation between serogroup Y and W135 amplicons using XbaI-specific RFLPs

Analysis of the restriction map of the serogroups Y and W135 siaD amplicon sequences revealed a XbaI restriction site unique to the serogroup Y siaD gene allowing discrimination between serogroup
Y and W135 meningococci. XbaI digests of serogroup Y siaD products produced the expected DNA fragments of 438 and 92 bp whilst the serogroup W135 siaD amplicon remained at 530 bp (Fig. 1) in agreement with expected size fragments from the restriction map of the serogroup Y and W135 sequences.

3.3. siaD PCR ELISA using serogroup-specific Y and W135 oligonucleotide probes

Amplification reactions were performed using boiled suspensions of N. meningitidis. All serogroup Y and W135 meningococci of diverse serotype or serosubtype examined were positive by siaD PCR ELISA for their respective serogroup as serologically determined with the exception of one serogroup W135 isolate. This isolate was serogroup Y positive by siaD PCR and upon regrouping gave positive reactions with both W135 and Y antisera. All meningococci of serogroups other than W135 or Y were negative on PCR ELISA. The serogroup Y and W135 probe sequences appear stable among 17 serogroup W135 and 15 serogroup Y meningococci of diverse phenotypes.

3.4. siaD PCR ELISA for detection of serogroup Y and W135 meningococci in clinical specimens

The initial evaluation shows that of the 83 clinical samples which were examined by the siaD PCR ELISA for serogroup Y and W135, 11 (13.3%) were positive for either Y or W135. Five samples (all CSF) were positive for serogroup W135, one of these samples having been collected from a culture confirmed case of W135 infection. Six samples (four...
CSF and two plasma) were positive for serogroup Y with three of these being collected from culture confirmed cases of serogroup Y infection.

3.5. Application of serogroup Y and W135 siaD PCR ELISA for improved epidemiological characterisation of non-groupable meningococci

A total of 27 boiled suspensions of non-groupable (NG) isolates, cultured from nasopharyngeal swabs during an epidemiological investigation following a community outbreak of meningococcal disease were examined. Three (11.1%) isolates were reactive in the serogroup Y and W135 PCR ELISA, two isolates were serogroup Y and one W135.

4. Discussion

In response to the increasing need for non-culture diagnostic methods for meningococcal infection, several PCR assays have been developed and described [4–9]. The development of the serogroup B and C siaD PCR ELISA [9] has proved particularly valuable due to the importance of serogroup determination for contact management. The introduction of meningococcal serogroups A and C conjugate vaccines may change meningococcal epidemiology, as reported for *H. influenzae* disease. The decline in the rate of infections by *H. influenzae* type b (Hib) following the introduction of the Hib conjugate vaccine has been accompanied by a quadrupling of infections due to *H. influenzae* type f in the United States from 0.5 case per 1000000 population in 1989 to 1.9 cases per 1000000 population in 1994 [14]. The implementation of vaccination with meningococcal serogroups A and C conjugate vaccines may have a marked impact on the carriage and disease caused by meningococcal serogroups, particularly since meningococci have a high potential for genetic transformation.

The effect of the currently licensed serogroup A and C polysaccharide vaccine on nasopharyngeal carriage in Italian army recruits has already illustrated that an increase in serogroups, other than those contained within the vaccine, may occur [15]. In this particular study throat swabs were collected at time of vaccination and again three weeks later. At the time of the first survey, the overall carriage rate was 32% with 17% of these belonging to serogroup Y but at the time of the second survey the overall carriage rate had reached 52% with serogroup Y accounting for 40%. No isolate at the time of the second survey belonged to serogroups A and C, however, this inhibition of A and C is offset by an increased prevalence of serogroup Y meningococci [15].

The siaD PCR ELISA described in this report is specific for serogroup Y and W135 meningococci, however, a single serogroup W135 meningococcal isolate, as determined using antisera, was amplified and found to react with the serogroup Y probe. Upon re-serogrouping with the W135 and Y antisera, it was discovered that this particular meningococcus agglutinated with both antisera. Problems with cross-reactivity between the W135 and Y antisera are common due to the biochemical similarity of these polysaccharides.

Meningococci can undergo down regulation of capsular expression, particularly following nasopharyngeal colonisation and establishment of the carrier state [16]. This results in organisms, non-groupable by conventional serological methods which are frequently isolated from healthy carriers. The identification of the serogroup status of organisms isolated from healthy contacts during outbreak investigations will enable more accurate identification of outbreak strain carriage, providing improved epidemiological information for management and infection control. Of the phenotypic markers used for the epidemiological characterisation of meningococci, the serogroup is of greatest importance for contact management because of the current availability of licensed A, C, W135 and Y vaccines. In the absence of clinical isolates, outbreak management is hindered. The implementation of outer membrane protein and polysaccharide-protein conjugate vaccines for serogroup B and C meningococci, respectively, requires maximum case ascertainment and serogroup identification to determine the true incidence of disease and thereby accurately determine efficacy.

This study has demonstrated a useful test for the detection of serogroup Y and W135 meningococci by the siaD PCR ELISA assay for the non-culture diagnosis and confirmation of meningococcal infection. The siaD PCR assay additionally provides a
timely identification of meningococcal serogroups allowing the effective implementation of appropriate methods for contact management and infection control. The addition of the serogroup-specific Y and W135 PCR ELISA assay for the non-culture confirmation of meningococcal infection in England, Wales and Scotland will be able to provide a more complete determination of the levels of serogroup Y and W135 infection prior to the introduction of conjugate vaccines into the childhood immunisation schedule.

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References