Evaluation of the Applied Biosystems automated Taqman polymerase chain reaction system for the detection of meningococcal DNA

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Abstract

In a period where the proportion of culture confirmed cases in the UK has been steadily declining, diagnosis by PCR has been used to increase the number of confirmed cases and provide additional epidemiological data. This report presents a comparative evaluation of the fluorogenic probe-based 5′ exonuclease assay (Taqman) using the Perkin-Elmer Applied Biosystems automated sequence detection system 7700 with previously reported polymerase chain reaction enzyme-linked immunosorbent (PCR ELISA) assays for the detection of meningococcal DNA in CSF, plasma and serum samples. Taqman assays developed were based on the detection of a meningococcal capsular transfer gene (ctrA), the insertion sequence IS1106 and the sialytransferase gene (siaD) for serogroup B and C determination and compared with similar assays in a PCR ELISA format. The Taqman ctrA assay was specific for Neisseria meningitidis, however the IS1106 assay gave false positive reactions with a number of non-meningococcal isolates. Sensitivity of the Taqman ctrA, IS1106 and siaD assays testing samples from culture-confirmed cases were 64, 69 and 50%, respectively, compared with 26, 67 and 43% for the corresponding PCR ELISA assays. Improvements to the DNA extraction procedure has increased the sensitivity to 93 and 91% for the TaqMan ctrA and siaD assays, respectively, compared to culture only confirmed cases. Since the introduction of Taqman PCR a 56% increase in laboratory confirmed cases of meningococcal disease has been observed compared to culture only confirmed cases. The developed Taqman assays for the diagnosis of meningococcal disease enables a high throughput, rapid turnaround of samples with considerable reduced risk of contamination. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Meningococcal disease; Non-culture; Taqman polymerase chain reaction; Polymerase chain reaction enzyme-linked immunosorbent assay

1. Introduction

The discrepancy between totals of meningococcal disease cases notified to the Office of National Statistics for England and Wales, which includes clinical diagnosis alone and culture-proven meningococcal disease cases has increased in recent years [1]. This observation is due in part to the increasing use of pre-admission antibiotics and the greater circumspection about performing lumbar puncture. In response, the Public Health Laboratory Services (PHLS) Meningococcal Reference Unit (MRU) based at Withington Hospital, Manchester, UK introduced in 1992 serological assays that provide useful but retrospective evidence of meningococcal infection [1]. Non-culture polymerase chain reaction (PCR) assays have been described that can amplify meningococcal sequences direct from clinical samples [2–10], with some assays providing additional information about epidemiological markers such as the serogroup [2], serotype [10] and serosubtype [9].

Developments of assays in a PCR enzyme-linked immunosorbent assay (ELISA) format using internal oligonucleotide probes have improved sensitivity and specificity as well as allowing more rapid processing of samples in a microtitre plate format [2,3,10]. The development of PCR ELISA enabled the MRU to provide a non-culture service for the laboratory confirmation of meningococcal infection. However, the post PCR processing involved is
lengthy and despite the inclusion of uracyl-N-glycosylase (UNG) manipulation of PCR products provides a potential source of contamination. Following the introduction of PCR testing as a national reference service for meningococcal case confirmation in 1995–1996 the demand in terms of numbers of samples submitted rapidly became overwhelming. With the level of throughput it became impossible to maintain the expected turnaround times using the PCR ELISA format. The Perkin-Elmer Applied Biosystems (PE-ABI) 7700 automated PCR platform employing Taqman chemistry was evaluated as a solution to this problem. The ABI sequence detection system employs fluorescence-based closed tube format that eliminates post PCR processing and provides accurate real-time quantitative PCR. Taqman assays have been described for the detection of several pathogenic organisms including hepatitis C [11,12], Salmonella spp. [13], Listeria monocytogenes [14] toxigenic Escherichia coli [15] and Mycobacterium tuberculosis [16]. The Taqman assay utilises a dual-labelled fluorescent probe, the 5′ end of which is labelled with one of several reporter dyes such as FAM (6-carboxyfluorescein) and the 3′ end with the fluorescent dye TAMRA (6-carboxy-tetramethylrhodamine) [17–20]. The spatial proximity to the reporter dye of the probe quenches the fluorescent emission. During amplification, the probe hybridises to the target sequence bound by the primers and is subsequently digested by the 5′ exonuclease activity of Taq DNA polymerase during primer extension releasing the reporter dye resulting in a relative increase in fluorescent signal. Automated monitoring of the fluorescent signal is possible using the PE-ABI 7700 sequence detection system during thermal cycling. Thus, no post PCR-processing of amplified products is necessary. The monitored reporter signal for a reaction is identified as positive when the fluorescent signal exceeds a background threshold level. Amplification at this point is exponential and has been shown to be the most reliable approach to quantification of input nucleic acid [17,18].

This study reports the evaluation of the fluorogenic 5′ nuclease assay (Taqman) for the detection of Neisseria meningitidis DNA from whole blood, plasma, serum and cerebrospinal fluid (CSF).

2. Methods

2.1. DNA extraction

2.1.1. Bacterial strains

N. meningitidis isolates used were obtained from the MRU culture collection. Meningococcal serogrouping and serotyping were carried out as previously described [21,22]. Other Neiserial species were obtained from the National Collection of Type Cultures (PHLS, Colindale, London, UK), Streptococcus pneumoniae, Moraxella catarrhalis, Staphylococcus aureus and Haemophilus influenzae cultures used were clinical isolates. DNA was extracted from overnight cultures grown on blood agar at 37°C in 5% CO2. Cultures were suspended in water and adjusted to approximately 2000 cfu 100 μl−1. Gram-negative bacteria were boiled for 15 min and centrifuged for 10 min at 12000×g. The supernatant was used as a PCR template and inoculated into the PCR reaction mix. Gram-positive bacterial DNA was extracted by adding 100 μl of bacterial suspension to 1 ml of DNAzol® solution (Life Technologies, Glasgow, UK), which was then vortexed and incubated for 10 min at room temperature. DNA was precipitated by adding 0.5 ml ethanol and centrifuging for 10 min at 12000×g. The pellet was washed with 1 ml of ethanol and finally resuspended in 50 μl sterile water.

The specificity of the Taqman ctrA and IS1106 assays was assessed by amplifying extracted DNA from other Neisserial species Neisseria elongata (N = 1), Neisseria pharyngis (N = 1), Neisseria lactamica (N = 10), Neisseria gonorrhoeae (N = 1), Neisseria flavescens (N = 1) and M. catarrhalis (N = 5) and from clinical and carrier isolates of meningococcal serogroups B (N = 11), C (N = 11), Y (N = 6), W135 (N = 3), A (N = 3), X (N = 7), 29E (N = 3), Z (N = 4) and H (N = 1). Other organisms known to cause meningitis were also examined and included S. pneumoniae (N = 15), S. aureus (N = 11) and H. influenzae (N = 4).

2.1.2. Clinical samples

Specimens of CSF, serum and plasma from culture proven and suspected meningococcal infection, were referred to the MRU from microbiology laboratories throughout England and Wales. Culture was attempted for all CSF extracts were used directly in PCR reaction mixes.

A subsequent evaluation of whole blood extractions was carried out using the Gentra DNA extraction kit (Flowgen, Lichfield, UK). A total of 216 consecutive EDTA blood samples from cases of meningococcal disease confirmed by blood culture were extracted and tested by TaqMan® ctrA and siaD B and C assays. EDTA whole blood samples (200 μl) were extracted using Gentra DNA extraction columns according to the manufacturer’s instructions.

2.1.3. Primers and probes

Oligonucleotide primers and probes were used to detect the IS1106, ctrA and siaD gene sequences in PCR ELISA and Taqman assays are shown in Table 1. Primers and probes used for the Taqman assays were synthesised by PE-ABI (Warrington, UK) and were designed using Primer Express software package (PE-ABI). Taqman probes were synthesised with the reporter dye FAM covalently
linked to the 5’ ends and the quencher dye TAMRA at the 3’ends which were phosphorylated to prevent probe extension.

2.2. PCR ELISA

2.2.1. DNA amplification

Aliquots (5 μl) of extracted samples were added to a 45-μl volume of PCR reaction mix comprising of 2 units of Taq DNA polymerase (Life Technologies, Glasgow, UK), 2 mM MgCl₂, 5 μl of 10× buffer, 0.2 μM of each primer, 200 μM dATP, dGTP, dCTP, 50 μM dTTP, 150 μM dUTP, 10 μM digoxigenin-11-dUTP and 2 units of UNG (Boehringer Mannheim, Lewes, UK). Reaction mixes were incubated at 40°C for 10 min followed by 95°C for 10 min. Thermal cycling was carried out using an MJ PTC 200 thermal cycler (GRI, Braintree, UK) and cycling conditions were as follows: IS1106 assay (94°C for 25 s, 63°C for 40 s, 72°C for 60 s), ctrA assay, (94°C for 25 s, 56°C for 30 s, 72°C for 30 s) and for siaD B and siaD C assays (94°C for 60 s, 55°C for 40 s, 72°C for 60 s). Cycles were repeated 40 times for all assays with a final extension step of 5 min at 72°C.

2.2.2. ELISA detection

This was carried out according to the manufacturer’s instructions (Boehringer Mannheim, Lewes, UK). Digoxigenin-labelled PCR products were hybridised in solution to sequence specific biotin-labelled probes. Hybridised probes were immobilised on to streptavidin coated plates and subsequently detected by anti-digoxigenin peroxidase conjugate and the substrate ABTS. Optical density values at 450 nm were recorded. For each assay, negative plasma extraction controls were included after every fifth sample. Plasma containing 2000 cfu 100 μl⁻¹ of a cultured isolate of N. meningitidis was included in every test as an extraction control. The cut-off was defined as twice the mean of the negative controls.

2.2.3. Amplification using the PE-ABI 7700 sequence detection system

A volume of 2 μl of extracted samples was added to 23 μl of PCR reaction mix prepared from Taqman Core Reagent kit (PE-ABI) which comprised 0.12 units of AmpliTaq Gold, 6 mM MgCl₂, 200 μM of each of dATP, dCTP, dGTP and dUTP, 0.25 units of uracil-N-glycosylase, 200 μM of each of two primers and 100 μM of FAM-labelled probe. DNA amplification was carried out on the PE-ABI 7700 sequence detection system by heating at 50°C for 5 min, 95°C for 10 min followed by 45 cycles of a two-stage temperature profile of 95°C for 15 s and 60°C for 1 min. Data points collected following primer extension were analysed at the end of thermal cycling. A threshold value is determined as 10 S.D. above the mean of the background fluorescence emission for all wells between cycle 1 and 15. The cycle number at which the fluorescence signal from a positive sample crosses this threshold is recorded.

2.2.4. Detection of PCR inhibitors

The PE-ABI exogenous internal control was used to determine if PCR inhibitors were present in the DNA extracts. Reagents are provided as a prepared kit which can be added to the TaqMan® reaction mix described above. The kit contains control target DNA, a VIC® fluorescently labelled probe and appropriate primers. Concentration of the primers is reduced to prevent competition with the sample target DNA for PCR reaction components. Analysis of the amplified internal control was carried out using the TaqMan® dedicated software which can resolve the FAM fluorophore used to detect meningococcal PCR product and the VIC® fluorophore for detection of the internal control PCR product.

An assessment of the presence of PCR inhibitors was carried out by amplifying 94 consecutive samples extracted by Gentra and by boiling using the TaqMan® ctrA assay.

2.3. Generation of a quantitative standard

The PCR product generated from primers amplifying part of the ctrA gene was cloned using the Invitrogen TOPO (R&D systems Oxford, UK) cloning system. From a selected plasmid containing the cloned ctrA PCR product a purified plasmid preparation was obtained (Pureprep Kit, Pharmacia, Milton Keynes, UK). The optical density of the plasmid preparation was determined spectrophotometrically (GeneQuant, Pharmacia) and the DNA concentration calculated. Titration of this standard was used to determine the sensitivity of the ctrA assay by measuring the limit of detection.

3. Results

3.1. Specificity of IS1106 and ctrA PCR ELISA and Taqman assays

Isolates of N. meningitidis were selected on the basis of genotypic and phenotypic diversity. Other Neisserial species and meningitis causing pathogenic bacteria were tested by both IS1106 and ctrA Taqman assays. The PCR ELISA and Taqman IS1106 assays detected all the meningococcal isolates, however, two of the ten S. pneumonie isolates, one of the five M. catarrhalis isolates and seven of the ten N. lactamica isolates were also amplified by the Taqman assay (Table 2). The PCR ELISA and Taqman ctrA assays were negative for all non-meningococcal isolates tested. All serogroup B, C, Y and W135 meningococcal isolates were positive, however, the non-sialic acid containing capsular serogroup organisms A, X, Z and 29E were not detected because of nucleotide sequence variation within the 5’ end of the ctrA gene [23].
3.2. Specificity of the serogroup B and C siaD Taqman assay

Serogroup B and C meningococci of a diverse range of serotypes and serosubtypes were tested. As reported for equivalent PCR ELISA assays [2], the Taqman siaD B assay detected exclusively serogroup B meningococci and the siaD C assay selectively identified only serogroup C meningococci. All other serogroups were negative by both siaD B and C assays.

3.3. Detection limit of Taqman assays

Tenfold dilutions of meningococci were prepared in pooled CSF plasma and serum negative for meningococcal DNA by PCR. From viable counts at each dilution, all four Taqman assays were able to detect less than one viable organism. In addition, titration of the cloned ctrA PCR product showed the Taqman assay to have a detection limit of two plasmid copies.

Table 1

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Forward primer 5'-3'</th>
<th>Reverse primer 5'-3'</th>
<th>Probe 5'-3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) PCR ELISA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ctrA gene</td>
<td>ATGCCGTTGCTGCGGTAGGT (744–763)</td>
<td>CCGGCCGAAACAAACGACAAAG (1277–1255)</td>
<td>TGGGTGCGGTGATGTTGGTT (873–892)</td>
</tr>
<tr>
<td>IS1106</td>
<td>ATTATTCAGACCGCCGCCAG (850–869)</td>
<td>TGCCGTCCGCAACTGATGT (1180–1161)</td>
<td>GTACGGTGGCGGAAAGCCTAT (1001–1018)</td>
</tr>
<tr>
<td>siaD B</td>
<td>CTCCTACCCCTACCAATACTGTCC (4139–4160)</td>
<td>TGGCCGCGGAAATAGTAAATAATGTT (4595–4572)</td>
<td>CAAATGTGGAAACACTGAAATG (4203–4225)</td>
</tr>
<tr>
<td>siaD C</td>
<td>GCACATTACGGCGGGATTAG (643–662)</td>
<td>TCTCTTGGTTGGCCTGATGTTGTA (1084–1061)</td>
<td>TGGACTGACATGCATTCTATTGG (972–994)</td>
</tr>
<tr>
<td>(B) Taqman assays</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ctrA gene</td>
<td>TGTGTGAGTTGTTATGTTGAGATGCT (185–211)</td>
<td>TCAGATTTGTTGCCCTAAGAGCACA (273–250)</td>
<td>TCCTTCACGGCCCCCAGCG (220–239)</td>
</tr>
<tr>
<td>IS1106</td>
<td>AGGCTATACCGGAAACACTGCACA (1014–1036)</td>
<td>TGGCCGACGTCTTCCCAAACA (1097–1079)</td>
<td>CAATGCCCATGAGTGCAAGACCTG (1047–1071)</td>
</tr>
<tr>
<td>siaD B</td>
<td>TGATGCTTCCCCTCTCCTGA (3708–3726)</td>
<td>AATGGGTTACAGTTGACTAACA (3877–3855)</td>
<td>TGCTTATCCCTCAGATGCGCAA (3794–3770)</td>
</tr>
<tr>
<td>siaD C</td>
<td>GATAATTTGGATATTTTGGCTAGTGGCCT (562–591)</td>
<td>TGAGATATGCCGATTGCTCTGGAT (710–685)</td>
<td>TGCGGGTGCTCTATGCCTGCA (672–649)</td>
</tr>
</tbody>
</table>

Genbank accession codes for ctrA, IS1106, siaD B and siaD C are M57677, Z11857, M95053 and U75650, respectively. Probe 5'-3' is 5' biotin-labelled for PCR ELISA and 5' FAM-labelled for Taqman probes. Nucleotide positions are shown in brackets.

Table 2

Specificity of the Taqman versus PCR ELISA

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of isolates</th>
<th>Taqman IS1106 positives (%)</th>
<th>Taqman ctrA positives (%)</th>
<th>PCR ELISA IS1106 positives (%)</th>
<th>PCR ELISA ctrA positives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumoniae</td>
<td>15</td>
<td>2 (13)</td>
<td>0 (0)</td>
<td>1 (6.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>M. catarrhals</td>
<td>5</td>
<td>1 (20)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>11</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>4</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>N. elongata</td>
<td>1</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>N. pharyngis</td>
<td>1</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>N. lactamica</td>
<td>10</td>
<td>7 (70)</td>
<td>0 (0)</td>
<td>7 (70)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>N. gonorrhoeae</td>
<td>1</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>N. flavescens</td>
<td>1</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>E. coli K1</td>
<td>1</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>N. meningitidis serogroup B</td>
<td>10</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
</tr>
<tr>
<td>N. meningitidis serogroup C</td>
<td>11</td>
<td>11 (100)</td>
<td>11 (100)</td>
<td>11 (100)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>N. meningitidis serogroup Y</td>
<td>6</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>N. meningitidis serogroup W135</td>
<td>3</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>N. meningitidis serogroup A</td>
<td>3</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>N. meningitidis serogroup X</td>
<td>7</td>
<td>7 (100)</td>
<td>0 (0)</td>
<td>7 (100)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>N. meningitidis serogroup 29E</td>
<td>4</td>
<td>4 (100)</td>
<td>0 (0)</td>
<td>4 (100)</td>
<td>4 (100)</td>
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<tr>
<td>N. meningitidis serogroup Z</td>
<td>4</td>
<td>4 (100)</td>
<td>0 (0)</td>
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<td>4 (100)</td>
</tr>
<tr>
<td>N. meningitidis serogroup H</td>
<td>1</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td>1 (100)</td>
<td>1 (100)</td>
</tr>
</tbody>
</table>
3.4. Comparative sensitivity of PCR ELISA and Taqman assays with clinical samples

Clinical specimens (N = 44), including CSF, plasma and serum samples from patients with suspected meningococcal disease positive by both IS1106 and ctrA PCR ELISA assays were also positive by the Taqman ctrA assay, however only 41 (93%) were positive by the Taqman IS1106 assay. Of the 32 selected clinical samples that were IS1106 PCR ELISA positive and ctrA PCR ELISA negative, the Taqman IS1106 assay detected only 19 (59%) and the Taqman ctrA assay detected 29 (91%) (Table 3).

Comparative sensitivities for the PCR ELISA and Taqman assays with samples from culture confirmed cases showed an improved sensitivity for all four Taqman assays (Table 4). Of 42 samples including CSF and serum or plasma samples tested by the Taqman assay, 29 (69%) were positive by the IS1106, 27 (64%) were positive by the ctrA assay and 21 (50%) were positive in either the siaD B or C assays compared with 28 (67%), 11 (26%), 18 (43%) positive by the PCR ELISA IS1106, ctrA and siaD B or C assays respectively. The 21 samples for which a serogroup was determined by Taqman represented 72% and 78% of Taqman IS1106 and ctrA positive samples respectively, and for the PCR ELISA the 18 serogroup positive samples represented 64% of the IS1106 positives. The ctrA PCR ELISA was less sensitive than the siaD assays detecting only 26% of the culture confirmed cases. All serogroup determinations by Taqman assay were also positive by the ctrA or IS1106, or both. PCR ELISA serogroup results were concordant with the Taqman assays.

The sensitivity of the Gentra extracted whole blood samples was compared with blood culture confirmed cases, and of 216 samples tested 201 (93%) were positive by TaqMan® ctrA and 196 (91%) were positive in either the siaD B or C assays.

Table 4
Comparative sensitivities for the PCR ELISA and Taqman assays with samples from cultured confirmed cases

<table>
<thead>
<tr>
<th>Taqman positive sample (%)</th>
<th>PCR ELISA positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS1106</td>
<td>29/42 (69)</td>
</tr>
<tr>
<td>ctrA</td>
<td>27/42 (64)</td>
</tr>
<tr>
<td>siaD (B and C)</td>
<td>21/42 (50)</td>
</tr>
</tbody>
</table>

3.5. Impact on sample processing

A maximum of 50 samples could be processed in a working day using the PCR ELISA system taking approximately seven hours to complete. Using the ABI 7700 automated platform with a 96-well plate format it was feasible to process 200 samples per day, representing a four fold increase in throughput compared to the PCR ELISA format. Turnaround times were also reduced with same day results available for the screening assay and serogroup results available the following morning. In addition, identical thermocycling conditions for all Taqman assays allowed both ctrA and siaD assays to be run in the same plate.

4. Discussion

The development of PCR assays for the non-culture detection of meningococcal DNA has enabled confirmation of cases, where in particular, early antibiotic treatment precludes detection by culture. In addition the detection of meningococcal DNA from peripheral blood by PCR [2,3,6,10] encourages clinicians to submit an easily accessible sample without the discomfort and risks associated with lumbar puncture. The introduction of PCR assays for the non-culture confirmation of meningococcal infection has markedly increased the number of confirmed cases. Analysis of data from the MRU showed that an additional 543 cases were identified by PCR alone between July 1996 and June 1997 which represented a proportional increase of 35% of the number of culture confirmed cases [24]. For 1998 an additional 871 cases have been confirmed by PCR alone from a total of 2445 laboratory confirmed cases, which represents an increase of 56% of the culture confirmed cases and 36% of the total number.

Specificity of the Taqman ctrA assay was comparable with the PCR ELISA ctrA assay, but showed an increased sensitivity most likely due to improved primer design. Sensitivity of the Taqman ctrA was comparable to the PCR ELISA IS1106 assay and titration of the cloned ctrA product in the Taqman assay was close to the theoretical limit with reproducible detection of two plasmid copies. The ctrA Taqman assay, however does not amplify serogroup A, X, 29E, Z, and H meningococci due to sequence var-
Transposable insertion elements are attractive targets for PCR assays since they are present as multiple copies in the genome, however the inherent genetic mobility results in their spread to other organisms [25,26]. This therefore reduces the diagnostic utility of the IS1106 as a target for confirmation of meningococcal disease. The improved sensitivity of the meningococcal specific ctrA PCR provides a more specific alternative to the IS1106 as a target for confirmation of meningococcal infection.

The sensitivity of all PCR assays tested compared to culture confirmed cases was at best 69% (Table 4), however, CSF was not always available for testing and of 13 PCR negative samples five plasma samples were examined where the isolate was cultured from CSF. The sensitivity of CSF samples may also be compromised as PCR examination is usually performed from centrifuged supernatant of CSF samples previously examined by microscopy and culture by the referring laboratory. The Taqman siaD B and C assays were specific for the respective sequence and more sensitive than their PCR ELISA equivalent assays. In both assay formats, the siaD B and C assays were less sensitive than the corresponding ctrA and IS1106 assays and may reflect constraints on available sequence for discriminatory primer design. Substances inhibitory to PCR amplification can result in false negative results, however, both boiled and Gentra-extracted samples were shown not to contain inhibitors. Evaluation of the Gentra whole blood extracted samples showed a marked improvement in sensitivity (93%) when compared to blood culture confirmed cases. Differences in the sensitivity between the two extraction methods were therefore due to differences in the efficiency of the procedures. The aim of this evaluation was however to compare the performance of the PCR ELISA and TaqMan® platforms which was independent of the efficiencies of the DNA extraction procedure.

The determination of serogroup provides a valuable epidemiological marker for identification and management of both sporadic cases and outbreaks enabling confirmation of outbreaks and appropriate vaccination. Samples from culture confirmed cases of meningococcal disease tested by all four Taqman assays were more sensitive than their corresponding PCR ELISA assays and a high proportion of positives (78% of ctrA positives) were positive by Taqman serogroup siaD B and C assays.

Fluorescence-based Taqman chemistry combined with the PE-ABI 7700 was shown to provide a robust assay format. Standardised commercial reagents together with the 96-well format allows reproducible, high throughput of samples with assay times of 2 h for combined amplification and detection. In contrast to the PCR ELISA where sample processing takes 7–8 h, a four-fold greater throughput using the PE-ABI 7700 Taqman system can be achieved. The potential for high throughput of this system therefore allows a cost effective centralised approach to diagnostic PCR testing which justifies the initial capital outlay.

As a result of this evaluation the Taqman ctrA assay has been adopted as a single screening test followed by determination of serogroup by siaD B and C assays on all positive samples. Currently more than 12,000 samples are being examined annually with over half this total received in four months from December through March. Use of the Taqman system has enabled a same day screening and serogroup determination for confirmation of meningococcal disease by PCR to be made available as a national reference service for patients throughout England and Wales.

Real-time PCR provides an accurate and reproducible method for quantification by PCR [17,18]. The Taqman ctrA quantitative PCR data will be used in future work to assess the relationship between bacterial load and clinical outcome, which may provide important information regarding patient management.

References

porA from a single colony-forming unit of Neisseria meningitidis. Gene 137, 153–162.


