

# Performance characteristics of the polymerase chain reaction assay to confirm clinical meningococcal disease

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## Abstract

**Background**—Confirmation of clinical meningococcal disease (MCD) is essential for management of patients, contacts, and outbreaks. Blood and CSF cultures, the traditional gold standard diagnostic tests, have been adversely affected by preadmission parenteral penicillin and fewer lumbar punctures. Rapid, reliable serogroup determination without the need to grow isolates could improve laboratory confirmation of MCD.

**Aims**—To determine performance characteristics of the currently available meningococcal polymerase chain reaction (PCR) assays in a clinical setting.

**Methods**—Prospective study of 319 children presenting with a suspected diagnosis of MCD (fever and a rash, or suspected bacterial meningitis) over a 16 month period.

**Results**—A total of 166 (52% of all children had clinical MCD: diagnosis was confirmed microbiologically in 119 (72%) of these. Performance characteristics (sensitivity, specificity, negative predictive value, positive predictive value) in confirmation of clinical MCD were respectively (95% confidence interval): blood culture 31% (24–38%), 100%, 57% (49–65%), 100%; blood PCR 47% (39–55%), 100%, 65% (58–73%), 100%; any test positive 72% (65–79%), 100%, 77% (70–84%), 100%.

**Conclusions**—Meningococcal DNA detection in blood or CSF by PCR is a useful method of diagnosis of MCD. PCR of peripheral blood performs better than blood culture. In a child with clinically suspected MCD, PCR assays, bacterial antigen tests, and oropharyngeal swabbing for meningococcal carriage should be performed in addition to blood or CSF culture, to improve case confirmation.

(Arch Dis Child 2000;83:271–273)

Keywords: polymerase chain reaction assay; meningococcal disease; confirmation; performance characteristics

Meningococcal disease (MCD) remains an important cause of morbidity and mortality in children. Confirmation of suspected disease is helpful to clinicians in the management of individual cases, and is needed by public health physicians in the management of contacts and in outbreaks. Enhanced ascertainment will also serve to monitor the impact of the new conju-

gate serogroup C vaccine, which is currently being introduced to the childhood immunisation schedule.

A shift in general consensus policy over the last few years has meant that fewer lumbar punctures are now performed.<sup>1</sup> In addition, the increasing administration of prehospital penicillin has contributed to a significant fall in the number of culture confirmed cases of MCD.<sup>1</sup>

The widening gap between notified and culture confirmed cases has led to the development of non-culture based polymerase chain reaction (PCR) assays to detect meningococcal DNA directly from clinical specimens, thereby enhancing the confirmation of meningococcal disease. In 1996, a national PCR based service was established at the Public Health Laboratory Service (PHLS) Meningococcal Reference Unit (MRU) in Manchester, which resulted in a 35% increase of laboratory confirmed cases in the first year.<sup>2</sup>

The performance characteristics of a PCR–ELISA (enzyme linked immunosorbent assay) assay based on the sialyltransferase (*siaD*) gene sequence have been described, where performance was assessed against isolates of *Neisseria meningitidis*.<sup>3</sup> It showed the *siaD* PCR assay to have a specificity of 100% for all specimen types tested, a sensitivity of 81% for CSF, and a sensitivity of 63% for whole blood. Another study looked at the sensitivity and specificity of PCR on peripheral blood for the diagnosis of meningococcal disease in laboratory confirmed cases,<sup>4</sup> using a PCR–ELISA assay designed to amplify a segment of the insertion sequence *IS1106* found in nearly all meningococci. The sensitivity of this assay was 89% for serum and 100% for blood “buffy” coat.

This study, unlike the two previous studies which looked at laboratory confirmed cases, examines the performance characteristics of the currently available blood PCR assay in cases clinically diagnosed as probable MCD. This information is of greater value to clinicians who require confirmation of the disease in the absence of positive blood or CSF cultures, and who need to know whether blood and CSF PCR assays are reliable tests. It compares the sensitivity, specificity, negative, and positive predictive values of blood PCR versus blood culture, the current gold standard. In addition to comparing diagnostic performance of culture and PCR, this study sought to assess the reliability of the newly introduced assays.

## Patients and methods

A total of 319 children who presented between December 1997 and March 1999 with symp-

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Accepted 4 May 2000

Table 1 Comparison of performance characteristics of blood culture, blood PCR, and any test being positive

Test	Sensitivity (%)	Specificity (%)	Negative predictive value (%)	Positive predictive value (%)
Blood culture	31 (24–38)	100	57 (49–65)	100
Blood PCR	47 (39–55)	100	65 (58–73)	100
Any test positive*	72 (65–79)	100	77 (70–84)	100

\*Blood or CSF culture, blood or CSF PCR, blood latex antigen detection test, and throat swab culture (95% confidence intervals in brackets).

Table 2 Concordance between blood culture and blood PCR

	PCR positive	PCR negative	Total
Blood culture positive	34 (74%)	12 (26%)	46 (100%)
Blood culture negative	39 (35%)	71 (65%)	110 (100%)
Total	73	83	156

toms suggestive of meningococcal infection were prospectively studied. In 166 children (probable cases), a clinical diagnosis of MCD was made in an ill child with a petechial or purpuric rash and a fever, or with signs of meningitis, who in addition may have had tachypnoea, tachycardia, impaired perfusion as manifested by a delayed capillary refill time, metabolic acidosis, and altered mental state. In 153 children (possible cases), an alternative diagnosis was made in a child who had a petechial or purpuric rash but was not ill, and did not show any sign of shock, so that the final diagnosis was not thought to be MCD. A total of 312 children had samples taken for blood or CSF PCR as soon as possible after admission, 279 had blood latex antigen detection tests, and all had blood cultures.

The MRU used two screening assays during the course of this study based on the *1106* insertion sequence<sup>4</sup> and the *ctrA* gene (submitted for publication). A third assay based on the sialyltransferase (*siaD*) gene sequence is able to discriminate between serogroups.<sup>3</sup> The sialyltransferase gene is involved in directing sialic acid synthesis (in the meningococcal capsule). The *siaD* PCR–ELISA assay is based on a stable restriction fragment length polymorphism (RFLP) in the *siaD* nucleotide sequence of serogroup B and C meningococci, identifying and distinguishing between these serogroups. It is used on specimens positive on either or both screening tests, but as it is less sensitive than the screening assays, not all specimens positive on screening will confirm a serogroup. The laboratory diagnostic test changed from a PCR–ELISA format to a Taq-Man (ABI, Warrington, UK) based system during the course of this study. Robustness and reliability were improved while overall performance remained unaltered.<sup>2</sup>

The specificity of the test was determined using the patients categorised as possible cases. Data on all microbiological results were recorded on a specially designed proforma. The study protocol was approved by the Local Research Ethics Committee. Parental informed consent was obtained before inclusion of children into the study.

## Results

There were 187 boys (59%) and 132 girls (41%). The median age was 2.23 years,

interquartile range (IQR) 0.9–6.7 years. In the 166 children with clinically diagnosed MCD, the diagnosis was confirmed microbiologically in 119 (72%). Blood or CSF culture was positive in 57/166 (34%), blood or CSF PCR in 82/159 (52%), and blood antigen tests in 32/146 (22%). Diagnosis was confirmed by blood culture alone in 15 (9%), PCR alone in 28 (17%), while in 59 (36%) the diagnosis was confirmed on more than one test. Eighteen had CSF culture performed, of which six were positive. CSF samples from 14 cases were sent for PCR of which nine were positive.

Table 1 presents a comparison of performance scores in clinically diagnosed MCD. The number of patients with any test positive (blood or CSF culture, blood or CSF PCR, blood latex antigen detection test, and throat swab) is included in the table, as it highlights the fact that performing all these tests in a child with probable MCD increases diagnostic yield.

In the children with a diagnosis other than MCD (possible cases), 133 (87%) had blood antigen tests performed and 152 (99%) had blood PCR; all had blood culture. None of these tests were positive. None of the children with a diagnosis other than MCD had CSF PCR or culture performed, and therefore we were unable to determine the specificity of CSF culture and CSF PCR.

Table 2 shows the concordance between positive blood culture and positive blood PCR. In those with a positive blood culture, 74% were blood PCR positive, and in those with negative blood cultures, 35% were blood PCR positive. In those with a positive blood PCR, 47% were blood culture positive, and in those with negative blood PCR, 86% were blood culture negative ( $\chi^2$  test,  $p < 0.0005$ ). There was a high degree of concordance between blood culture and blood PCR, but PCR confirmed more cases than did blood culture.

## Discussion

This study shows the value of the PCR assay for the detection of meningococcal DNA. The assays were found to be sensitive and highly specific. There were no false positives in this series, although they have been described with the assay for IS1106.<sup>5</sup> The results in our study are consistent with those of Borrow *et al* who found that in specimens from culture negative cases of clinically suspected MCD, 31% were positive by blood PCR.<sup>3</sup>

This is the first study to look at the performance characteristics of the PCR assay in clinically diagnosed disease (probable cases). It shows that the current gold standard for the confirmation of clinically diagnosed disease, blood culture, is a less sensitive test with a higher false negative rate than PCR in blood. In 14% of children with positive blood culture, meningococcal DNA was not detected by blood PCR. This may be a reflection of the small quantity of clinical sample (2  $\mu$ l) employed in the PCR assay compared to blood culture. Specimens for PCR should be taken as soon as possible after admission, otherwise this is likely to compromise diagnostic yield. While superior to culture alone, PCR of peripheral

blood provides suboptimal detection, and consequently could not be presented as a gold standard for confirming MCD. It is essential that both blood culture and blood PCR are performed on all children with clinically diagnosed MCD to increase the microbiological confirmation rate.

The 39 cases that were PCR positive but blood culture negative may have been false positives, but this is unlikely as they occurred in clinically diagnosed cases. They were notified to the Consultant in Communicable Disease Control (CCDC), and were treated as cases of MCD by the clinicians involved in their care, regardless of blood culture results. It may therefore be argued that performing blood PCR assays contributes little to the management of the individual child. While this may be true in practice, it must be emphasised that increasing confirmation of probable cases is extremely important for epidemiologists and consultants in public health. With the introduction of the new conjugate serogroup C vaccine, and while the development of effective serogroup B vaccines is awaited, a period of enhanced surveillance is necessary to monitor vaccine efficacy. This can only be achieved by optimising microbiological confirmation of clinically diagnosed cases.

In the clinical setting, an ill child presents to paediatricians with a fever and a rash, and in addition may have signs of impaired perfusion, acidosis, tachycardia, or tachypnoea. Performing lumbar puncture in a child with meningococcal septic shock may lead to an acute deterioration in clinical state secondary to the positioning of the patient. In addition, if there is raised intracranial pressure, and reduced cerebral perfusion pressure secondary to hypotension, lumbar puncture may result in acute brainstem herniation.

Even in the absence of laboratory confirmation, such children will usually be notified to the CCDC and treated as cases of MCD. In contrast to the obviously unwell child who presents with signs of shock, a significant number of relatively well children present to hospital with a petechial or purpuric rash, with or without pyrexia,<sup>6,7</sup> and it is this group of children that presents a dilemma for the clinician. Most would err on the side of caution, and treat such children with intravenous antibiotics while awaiting the results of blood culture, but the diagnostic yield in such cases is extremely low. The PCR assay is capable of yielding a result the same day, if the sample is received first thing in the morning, but in practice, it may take longer, depending on the arrangements used by the local microbiology laboratory in sending the sample to the MRU. Typically, clinicians receive a PCR result within three to four days, while the child is still in hospital.

Data from this study show that in children with a low index of suspicion (possible cases), blood PCR has a very low yield. As it is an expensive test to perform, this test should perhaps be reserved for probable cases. The PHLS MRU provides a national service for England and Wales, and particularly during the winter months, is faced with huge demands on the available resources. If this service is inundated with PCR requests from patients in whom the likelihood of a positive result is extremely low, the prompt reporting of results, which may influence clinical management and management of contacts nationally, may be compromised. Possible cases should have as a minimum, blood and throat swab culture performed. If uncertainties persist, retrospective serological confirmation using convalescent specimens can be used to resolve a proportion of these.<sup>8</sup>

#### CONCLUSION

PCR of peripheral blood is a useful test, and in nearly a fifth of clinically diagnosed cases, was the only positive test. Blood or CSF culture is currently regarded as the gold standard for the confirmation of MCD, but diagnostic confirmation is increased if, in addition, blood or CSF PCR tests, blood antigen, and throat swab investigations are performed. Performing PCR in possible cases gives a low yield. Our study shows that performing PCR assays of blood or CSF in probable cases of MCD will contribute significantly to the improved case ascertainment that is now urgently required.

We thank Dr Jeremy Fellick, Institute of Child Health, for seeing some of the patients on admission, the Infection Control Nurses, Mrs Julie Hughes and Mrs Doreen Brown and the technical staff of the Bacteriology Department, RLCH, Alder Hey for their contribution to the study, and the technical staff at the MRU, Manchester for providing the PCR results.

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