

## Specific PCR Assay for Rapid and Direct Detection of *Neisseria meningitidis* in Cerebrospinal Fluid Specimens

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### Abstract

**Background:** *Neisseria meningitidis* is one of the most frequently encountered microorganisms associated with central nervous system infections. The aim of this study was to evaluate a PCR-based assay for specific and rapid detection of *N. meningitidis* in CSF specimens.

**Methods:** Since April 2002 to July 2006, 130 CSF specimens were collected from patients suspected of having bacterial meningitis. Bacterial isolation and identification was carried out according to the standard bacteriological methods. The PCR was used to amplify a 101bp fragment of capsular transport gene A (*ctrA*) of *N. meningitidis*.

**Results:** PCR yielded an amplified product with the expected size of 101 base pair fragment. Sensitivity test proved 500 ng of *N. meningitidis* DNA as the final detection limit and specificity test revealed no cross-reaction for a wide range of respiratory pathogenic organisms.

**Conclusion:** The PCR assay was more sensitive than the bacterial culturing. It might be possible to apply this procedure for rapid diagnosis of meningococci in clinical samples.

**Keywords:** Meningococcal meningitis, PCR, *Neisseria meningitidis*, Rapid Detection

### Introduction

Meningococcal meningitis is a major cause of morbidity and mortality in all societies worldwide (1, 2). *Neisseria meningitidis* has been found as one the most common causes of bacterial meningitis among the individuals who live in the same place (3). Due to high morbidity caused by this disease, precise diagnosis and urgent treatment is necessary (4). The most common clinical symptoms for initial diagnosis of invasive meningococcal disease (IMD) included fever, vomiting, neck stiffness, and skin rash. The diagnosis of this disease is usually based on isolation of *N. meningitidis* from cerebrospinal fluid (CSF),

blood or other specimens and their biochemical reactions (5). In patients with IMD, particularly in cases which have been treated with antibiotics before collection of the samples, the results for culture are negative (6). The organism usually could be identified after culturing the CSF. Furthermore, antibiotics are prescribed routinely as a part of the pre-hospital management of bacterial meningitis. Consequently, this may make it difficult to culture the organisms (7). Besides, culturing requires a minimum of 8 to 12 h of incubation time before performing the biochemical and/or immunological tests to identify the bacterium. It has been shown that the time re-

quired to obtain a positive culture result can be even longer for the patients infected with slowly growing organisms or with low bacterial counts (8, 9). Therefore, the development and introduction of non-culture-based diagnostic methods such as the polymerase chain reaction (PCR) into clinical practice is necessary. For this reason, some studies have introduced PCR-based methods as good tools for the diagnosis of IMD (10-13).

The aim of this study was to evaluate a rapid PCR assay for direct detection of *N. meningitidis* in CSF samples.

## Materials and Methods

### *Bacterial strains and culture conditions*

The study was conducted from April 2002 to July 2006. The patients with suspected bacterial meningitis admitted to four military hospitals in Tehran, Iran were included in this study and underwent lumbar puncture. A total of 130 CSF samples were obtained and examined for the presence of *N. meningitidis* by both culture and PCR methods. Meningococcal strains were cultured under aerobic conditions with 3% to 5% CO<sub>2</sub> at 37° C on 5% sheep blood agar or chocolate agar supplemented with 0.5% glucose, 0.01% glutamine, and 0.5% yeast extract. *N. meningitidis* serogroup B 44/76 (B: 15:P1.7), (kindly provided by Dr. Bahman Tabaraie, Pasteur Institute of Iran), *N. meningitidis* ATCC 13090, *Escherichia coli* ATCC 35218 and *Haemophilus influenza* ATCC 49766 were used as reference strains for comparison purposes. Other clinical control strains were *Streptococcus pneumonia*, *Staphylococcus aureus*, *Streptococcus* group D and *Klebsiella pneumonia*.

### *PCR analysis*

The primers used in this study which were introduced previously (forward 5'-GTA-GGT-GGT-TCA-ACG-GCA-A-A-3' and reverse 5'-TCG-CGG-ATT-TGC-AAC-TAA-A-T-3') (Cinnagen, Iran), amplified capsular transport gene A (*ctrA*) (14).

Two or three colonies of bacteria were washed in 1 ml of distilled water (5000×g/5 min, at 4°C). One ml of TE buffer (1% Triton X-100, 10 mM Tris, and pH 8.0) was added to the pellets and vortexed. The suspensions were centrifuged at 12000×g for 5 min and the pellets were resuspended in 1 ml of the same lysis buffer and were boiled in a water bath for 30 min. The cell debris was removed by centrifugation at 5000×g for 5 min, and the supernatant was saved for PCR. To perform PCR on CSF samples, 500 µl of CSF was centrifuged at 12000×g for 5 min. The pellet was resuspended in 180 µl of sterile distilled water, and the DNA extracted as mentioned above. A PCR reaction mixture containing 50 ng of template DNA, 1X PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.001% gelatin), 0.3 mM of PCR primer, 0.2 mM of each deoxynucleoside triphosphate, and 0.5 U of *Taq* DNA polymerase (Nedaye Phan. Co. Tehran Iran) in a total volume of 50 µl was prepared. After a 10 min denaturation time at 94° C, the reaction mixture was run through 30 cycles of denaturation for 1 min at 94° C, annealing for 30 s at 62° C, and extension for 45 s at 72°C, followed by an incubation for 10 min at 72° C. Five micro liters of PCR product was electrophoresed on a 2% agarose gel to determine the size of the product. Both negative and positive controls were included in each PCR run. The PCR products were confirmed by sequencing (MWG, Germany). The sensitivity of amplification was tested with purified *N. meningitidis* DNA and bacterial cells count. The DNA was quantified spectrophotometrically. The concentration of DNA was calculated based on the facts that genomic DNA is absorbed in 260 nm and OD=1 equals 50 ng/ml.

## Results

PCR yielded an amplified product with the expected size of 101 bp fragment when the assay was carried out on the purified chromosomal DNA extracted from the standard and clinical strains of *N. meningitidis*. No amplified products were

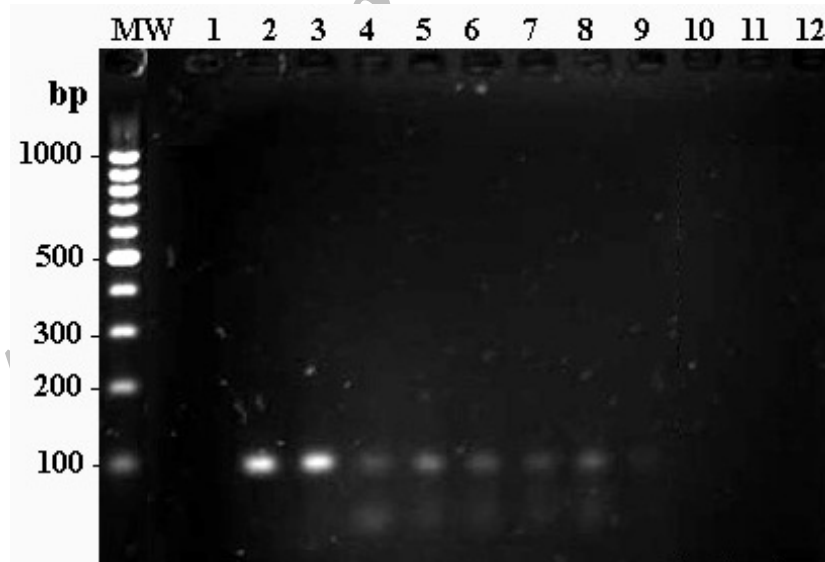
detected when the technique was performed on the strains belonging to different pathogenic organisms. Electrophoresis analysis revealed the lowest dilution of the *N. meningitidis* DNA that could be amplified by PCR was 50 ng of chromosomal DNA as the final detection limit, which is nearly 90 copies of the genome (Fig.1).

When comparing the assay with conventional laboratory methods (direct microscopic observation, and culture), amongst 130 CSF specimens, six and five cases (13.9%) had positive result by PCR and culture, respectively (Table 1). Clinical data from patients provided by public hospitals showed a low rate of antibiotic treatment before admission to hospital and CSF collection for laboratory analysis.

**Table 1:** Frequency of the results of cultures and PCR

Bacterial Species	No. of Bacterial Species	No. (%) of Positive Culture	Positive Results of PCR
<i>Neisseria meningitidis</i>	130 <sup>a</sup>	5 (13.9)	6
<i>Moraxella catarrhalis</i>	1 <sup>b</sup>	1 (2.7)	0
<i>Neisseria sica</i>	1 <sup>b</sup>	1 (2.7)	0
<i>Streptococcus pneumoniae</i>	13 <sup>b</sup>	13 (36.2)	0
Coagulase negative <i>Staphylococcus</i>	6 <sup>b</sup>	6 (16.7)	0
<i>Pseudomonas aeruginosa</i>	2 <sup>b</sup>	2 (5.6)	0
<i>Escherichia coli</i>	5 <sup>b</sup>	5 (13.9)	0
Gram positive bacilli	2 <sup>b</sup>	2 (5.6)	0
<i>Haemophilus influenza</i>	1 <sup>b</sup>	1 (2.7)	0
Total	161	36 (100)	6

<sup>a</sup> CSF specimens  
<sup>b</sup> Standard control strain



**Fig. 1:** PCR products shown on agarose gel. Lane MW, Molecular weight marker; lane 1, negative control; lanes 2-9, different concentrations of amplified DNA from *N. meningitidis*; lanes 10 – 12, CSF samples with no bacterial growth

## Discussion

Early diagnosis of bacterial meningitis is very important in effective and successful treatment of the patients. To achieve this goal an improved me-

thod with high sensitivity and specificity is needed (15).

Nowadays, different molecular methods have provided powerful tools for detection of important

virulence genes among the microbial pathogens and epidemiological studies of the diseases (16, 17). Microbiological studies are based on precise interpreting systems which could provide the keys for treatment of the diseases (18-21). Additionally, the molecular techniques could be useful especially for the detection of some fastidious pathogens (22).

As *N. meningitidis* is a fastidious organism which its culture and identification by conventional methods is difficult, it seems that development of rapid and sensitive DNA-based assays is necessary for direct detection of this bacterium in clinical specimens, especially cerebrospinal fluid specimens. Many studies have developed PCR methods in order to accurate and sensitive detection of *N. meningitidis* in some meningococcal meningitis cases but many of them are not readily available (23). The positive results for PCR assays has also been reported by PHLS Meningococcal Reference Unit, in the samples obtained from patients with culture negative results because of previous antibiotic treatment (24). On the other hand, several studies have shown that meningococcal DNA could be detected by PCR assay in CSF samples of patients with meningococcal meningitis (25).

In this study 50 ng of chromosomal DNA of reference *N. meningitidis* was the final detection limit for the test. However, a range of 2 fg to 5 pg has been reported for the sensitivity of PCR-based diagnostic methods for *N. meningitidis* in the clinical specimens (26). To validate an assay it should be compared with a reference standard, such as culture. Therefore, in this study after setting up and standardizing the PCR protocol, it was applied to clinical CSF specimens, and the results were compared with the results of the culture. The results showed that six CSF samples were positive for *N. meningitidis* DNA while 5 samples had positive result by culture. PCR method is probably more useful and applicable than the traditional gold standard, which was shown through this study, because nucleic acid amplification methods are inherently more sensitive. High sensitivity of PCR-based protocols has also been

reported by other authors when different DNA regions of the *N. meningitidis* genome were used for PCR amplification (27, 28). In present study, we observed that a set of *ctrA* primers is sensitive and specific enough to detect *N. meningitidis* DNA in clinical specimens. On the other hand, PCR test could overcome the difficulty of the culture method in diagnosis of *N. meningitidis* in cases with previous treatment by antibiotic. Our findings also showed that PCR could shorten the time of diagnosis of *N. meningitidis* in the clinical specimens to 3 h. However, the main goal of this study was to develop a rapid and sensitive PCR method to detect and identify *N. meningitidis* in CSF specimens.

In conclusion, this study showed that PCR assay was found to be more sensitive in diagnosis of meningococcal meningitis than culture method. Consequently, it may be possible to apply this procedure for rapid diagnosis of *N. meningitidis* directly in clinical samples of acute cases when culture is negative because of empiric antibiotic treatment.

## Ethical Considerations

All ethical issues including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc have been completely observed by the author.

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