Analytical Specificity and Sensitivity of a CrgA Gene Based PCR Assay for Rapid Detection of Neisseria meningitids Bacterium

Keivan Majidzadeh; Mohammad Soleimani; Amirhossein Mohseni*

Tasnim Biotechnology Research Center (TBRC), AJA University of Medical Sciences, Tehran, Iran

kmajidzadeh@razi.tums.ac.ir

Background & Objectives: *Neisseria meningitids* is one of the most important causative agents of the bacterial meningitis, a life-threatening disease in children and adults. There are several classic Methods for diagnosis of this bacterium but these are time consuming and unreliable. The goal of the present study was to evaluate of crgA gene for molecular detection of the organism.

Methods: The specific primers were designed for amplification of crgA gene and PCR reaction was setup using genomic DNA of *Neisseria meningitids* as template. In order to determination of the assay specificity, PCR were done on the other bacterial genomes via crgA specific primers. To create a standard positive control plasmid, the PCR product was cloned in the pTZ57R/T vector. The sensitivity of the assay was tested by preparation of 10 fold serial dilutions of positive control plasmid with starting concentration of 70 ng/ul. In order to better evaluate efficiency of the PCR assay in clinical situations, artificial contamination of normal CSF using the positive control plasmid was performed.

Results: The PCR reaction amplified a DNA fragment with expected size of 500 bp. The specificity evaluation indicated the specific primers of crgA gene don't have any amplification on genomic DNA of negative control bacteria.The sensitivity study showed 70 pg was the lowest concentration of the template which was amplifiable during the PCR. The PCR experiment on extracted DNA of artificially contaminated CSF showed amplification of a 500 bp fragment.

Conclusion: The study showed clearly the designed crgA gene based PCR is a specific and sensitive assay for molecular detection of *N. meningitids* in clinical specimens.

Keywords: PCR; *Neisseria meningitids*; Molecular Diagnostic; CrgA Gene