TITLE: CONSTRUCTION OF PLASMIDS FOR USE IN REAL TIME PCR ASSAYS FOR THE MULTIPLEX DETECTION OF BACTERIAL MENINGITIS PATHOGENS

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ABSTRACT:

Neisseria meningitidis (Men), *Streptococcus pneumoniae* (Spn), and *Haemophilus influenzae* (Hi) are common bacterial meningitis pathogens, and rapid, sensitive, and specific laboratory assays are essential for the implementation of appropriate antibiotic therapy in patient, and for the immediate interventions in control of outbreaks and epidemics of meningococcal disease. The multiplex qPCR assay for simultaneous detection of Men, Spn, and Hi standardized by our group has been widely implemented in other public health laboratories in Brazil and Latin America. To ensure the quality of qPCR assays it is necessary to have stable positive controls, reproducible and easy to produce to continuous distribution for this laboratory network. This study aimed to construct and obtain specific plasmids with inserts containing the genetic targets of qPCR multiplex assay to be used as positive controls for this test. Fragments of the genes *ctrA* (Men), *lytA* (Spn) and *hpd* (Hi) containing the target sequences of qPCR reactions and restriction sites for the enzymes BamHI, EcoRI and / or KpnI were cloned into the plasmid vector pUC18. The plasmids were expanded in *E. coli* cells DH5α and its extraction and purification was carried out using the Qiagen Plasmid Midi commercial kit. Cloned inserts had sequences according as described in GenBank for the three target genes. In qPCR standard curves, plasmids showed similar results to those presented by the genomic DNA: efficiency of 115.4% and r^2 of 0.996 for Men, 117.1% and r^2 of 0.999 for Spn and 121.1% and r^2 of 0.997 for Hi. The limits of detection were 10 copies/reaction for all plasmids. In the stability tests, all plasmids were stable for four months at three tested conditions (-20 ° C, 4 ° C and 24 ° C) with a maximum variation of 1 unit in the Ct value (Men, Ct = 21.9$\pm$0.4; Spn, Ct = 25$\pm$0.1; Hi, Ct = 31.8$\pm$0.9). The plasmids showed excellent stability and reproducibility in qPCR assay and represent an interesting tool to monitor the quality of this test in the public health laboratory network.

Keywords: bacterial meningitis, plasmids, real time PCR, molecular diagnosis

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