Title: CONSTRUCTION OF GENETIC CONTROLS FOR IMPROVING THE QUALITY OF REAL TIME PCR ASSAYS FOR BACTERIAL MENINGITIS DIAGNOSIS

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Abstract:

Bacterial meningitis remains a serious public health problem with a high rate of mortality and many cases of severe sequelae. Appropriate antimicrobial therapy should reduce the risk of dying from meningitis to below 15%, so it is important that treatment be started as soon as possible. For a rapid, sensible and specific diagnosis, we standardized in house real time PCR assays (qPCR) for detection of the main bacteria causing meningitis, which have been implemented in different public laboratories in Brazil and Latin America. This technology transfer has increased the demand for the positive controls used in these assays that were produced in laboratorial scale from bacteria grown in Petri plates. This procedure was impracticable for the production of large amounts of these controls due to biological hazards associated with the handling of highly infectious bacteria with epidemic potential. In this context, this study aimed to construct and obtain specific genetic controls (plasmids) that were used as positive controls for qPCR assays for the detection of *Neisseria meningitidis* (Men), *Streptococcus pneumoniae* (Spn) and *Haemophilus influenzae* (Hi), and the gene of human RNase P (RP), employed to verify the quality of DNA extraction process. In addition, we analyzed the stability of these plasmids in three different storage conditions (−20 °C, 4 °C and 24 °C) for a period of four months. Fragments of 1200, 844 and 1162 bp of *ctra* (Men), *lytA* (Spn) and *hpd* (Hi) genes containing the target sequences of qPCR reactions and restriction sites for the enzymes BamHI, EcoRI and/or KpnI were cloned into plasmid vector pUC18. RP control was obtained using a synthetic fragment of 530 bp cloned in pGEM-B1 vector. The plasmids were expanded in *E. coli* cells DH5α using the Qiagen Plasmid Midi kit for extraction and purification. The cloned inserts presented sequences in accordance with those described in GenBank for the genes *ctrA*, *lytA*, *hpd* and RP. In qPCR assays, plasmids showed expected results with appropriate amplification curves and multicomponent plots. All plasmids were stable for four months at the three tested conditions with maximum variation of one unit in Ct value (Ct = 21,9 $\pm$ 0,4 for Men; Ct = 25 $\pm$ 0,1 for Spn; Ct = 31,8 $\pm$ 0,9 to Hi and Ct = 31 $\pm$ 0,5 for RP). The use of specific plasmids as positive controls in qPCR assays improves the quality of these assays due to its greater stability and reproducibility as compared to bacterial suspensions controls.

Key-words: bacterial meningitis, real time PCR assay, genetic controls