Title: STANDARDIZATION OF MULTIPLEX QUANTITATIVE PCR ASSAY FOR PATHOGENIC BACTERIA ANALYSIS IN FOOD.

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Summary:

The consumption of contaminated food causes great concern and warning to the public health sectors. The search for analyzes that allow a rapid identification of pathogenic microorganisms is becoming increasingly necessary for monitoring the quality of food for human consumption. Quantitative real-time PCR (qPCR) can be used as an alternative analysis, as it allows quantifying bacterial numbers through DNA quantification during the exponential phase of the reaction. The aim of this study was to standardize a multiplex qPCR assay for *Salmonella* spp. (SL), *Staphylococcus aureus* (SA) and *Escherichia coli* (EC) quantifications, simultaneously, for further use in the monitoring of the microbial quality in food. Reference bacterial strains were acquired from Oswaldo Cruz Foundation (FIOCRUZ). DNAs from these microorganisms were extracted using commercial kit and subjected to a conventional PCR to amplify specific target genes in SL, EC and SA (*ST15* / *ST11*, *phoA* and *nuc* genes, respectively). Then, the PCR product was purified and quantified, and serial dilutions were performed to produce the standard curves with 10^6 to 10^0 copy numbers of each target gene. The multiplex qPCR was performed using MGB probes (Life Techologies) labeled with FAM, VIC and NED fluorophores to quantify SL, SA and EC, respectively. Primers and probes were specifically designed for this experiment. The standard curve of SL presented efficiency (% Eff) of 101,2% and a coefficient of determination (R^2) of 0.998; in SA, % Eff= 106,9% and R^2= 1; and in EC, % Eff= 99,461 and R^2= 0.998. Nevertheless, low sensitivity to detect and quantify EC was observed in the multiplex qPCR reaction, while SL and SA maintained the same sensitivity and efficiency in the multiplex reaction when compared with single reactions. Studies are still being conducted regarding the standardization of this multiplex qPCR reaction to improve the sensitivity EC quantification simultaneously with SL and SA quantifications.

Keywords: Quality control; Food safety; Foodborne diseases; Molecular diagnosis.

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