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Development and evaluation of novel PCR assays for the sensitive, rapid and accurate detection of *Salmonella spp., L. monocytogenes, E. coli* O157:H7 and *G. stearothermofillus* in pasteurized milk and fresh juice products

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Foodborne illnesses are related with high rates of morbidity and mortality as well as with substantial economic burden. The replacement of traditional and laborious protocols by sensitive and rapid methods is required, and PCR-based assays represent the corner stone of this effort. A multiplex PCR assay was developed for the *simultaneous* detection of *Salmonella spp., Listeria monocytogenes* and *Escherichia coli* O157:H7, using specific primers which target *rseB, prfA*, and *eaeA*, respectively. Quantitative TaqMan-based Real-time PCR (qPCR) methodology was developed for their quantification as well as for *Geobacillus stearothermofillus*. Pasteurized milk and fresh orange juice were spiked and cultured based to ISO methods. Samples were taken for DNA extraction and PCR analysis every two hours until 8 hours of culture. Assay specificity was individually assessed, using DNA from closely related species. Concerning singleplex qPCR, the detection limits for *Salmonella spp* and *G. stearothermofillus* were 5x10-4 ng DNA, while for *E. coli* O157:H7 and *L. monocytogenes* were $2.5x10^{-4}$ ng DNA. Using multiplex PCR assay, detection of the pathogens was succeeded following 4 hours of culture spiked with 10 cfu/ml or more, while accurate quantification of the pathogens was also performed for the same samples and all the spiked pathogens. We developed two specific and sensitive PCR assays for the simultaneous detection of three different food-associated pathogens, paving the way for their application in food analysis.

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