A REAL-TIME MULTIPLEX PCR ASSAY FOR THE DETECTION OF *SALMONELLA* ENTERITIDIS

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Introduction: Salmonellosis is an infectious disease caused by various serotypes of *Salmonella. Salmonella enterica* serotype Enteritidis and *Salmonella enterica* serotype Typhimurium are implicated in majority of *Salmonella* infections in Kazakhstan. For epidemiological studies, strain identification is necessary and such investigations have traditionally relied on biochemical and serological methods. Alternative method, such as real-time PCR can be used as an effective and reliable tool for detection of *Salmonella.* The aim of the study is to develop a real-time multiplex PCR assay for the detection of the *S.* Enteritidis serotype.

Methods: A total of 75 *Salmonella enterica* serotype Enteritidis and serotype Typhimurium strains were isolated at the Infectious diseases hospital (Astana, Kazakhstan) during 2016-2017. The isolation of DNA from inactivated bacterial cultures was carried out by phenol-chloroform extraction. The quantitative DNA content was carried out on a spectrophotometer (Nanodrop 1000) and evaluated by electrophoresis in a 1% agarose gel. Sequence-specific TaqMan probes and primers for the *invA* and *sefA* genes were designed using FastPCR 6.5 and Beacon Designer 8.0 software. All primer sequences were tested for complementarity using the database http://blast.ncbi.nlm.nih.gov/Blast.cgi. Real-time PCR was performed on a CFX96 (Bio-Rad) instrument. The fluorescence detection was set up after each annealing step and the results were analyzed using CFX Manager 2.1 software (Bio-Rad).

Results: The proposed assay is based on a duplex real-time PCR using competing TaqMan probes that are complementary to the nucleotide sequence of *S.* Enteritidis. The *invA* gene target is used for *Salmonella* detection, while *sefA* gene target sequence is specific for *S.* enteritidis. *InvA* gene is known to be in amplification control, while *sefA* gene encodes the fimbrial antigen SEF14 that is specific for *S.* enteritidis. All 75 *Salmonella* strains were positive for the *invA* target sequence. Fifty-one strains were positive for the *sefA* target sequence of *S.* Enteritidis (100% inclusivity, 95% exclusivity).

Conclusion: A duplex real-time PCR assay was developed for the detection of *S.* Enteritidis. The inclusivity and exclusivity were between 100 and 95% analyzing 75 bacterial strains.