Multiplex and quantitative pathogen detection using MLPA-CE-SSCP

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Diagnostics and disease-management strategies require technologies to enable the multiplex detection and quantification of a wide range of pathogenic microorganisms. However, most multiplex· quantitative detection methods available suffer from compromises between the level of multiplexing, throughput and accuracy of quantification.
Here, we demonstrate the novel multiplex pathogen detection method using capillary electrophoresis-single strand conformation polymorphism (CE-SSCP) coupled with multiplex ligation-dependent probe amplification (MLPA). MLPA-CE-SSCP is composed of four major steps which are probe hybridization, probe ligation, multiplex amplification and detection. First, MLPA probes hybridize to each species-specific target markers in a complex DNA sample. Second, probes which hybridize to specific target are ligated. Ligation step minimize false-positive of hybridization cause of ligation is carried out when MPLA probe exactly hybridize. After, multiplex amplification of ligated probes using common primer pair. Finally, the amplicons are separated and quantified by CE-SSCP analysis.