

Title Multiplex PCR for Simultaneous Detection of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* 0157:H7 in Meat Samples

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Abstract

The multiplex PCR system has been considered for the simultaneous detection of pathogens since the expense of reagents and the preparation time are less in multiplex PCR than in systems where several tubes are used. In this study, we describe the development of a multiplex PCR method capable of identifying *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* 0157:H7 directly from enrichment culture of meat samples. In addition, we have evaluated the optimization of pre-enrichment medium, DNA extraction method and the multiplex PCR setting. Detection sensitivity of this method showed that DNA from 10^3 CFU/mL of each pathogenic bacteria could be detected. When this protocol was used for the detection of each of the above pathogenic bacterium in spiked pork samples, 10^0 cells/25 g of inoculated sample could be detected within 30 h. Also in the samples of naturally contaminated meat, *Salmonella* spp., *L. monocytogenes*, and *E. coli* 0157:H7 were detected by the same time period. Excellent agreement of the results of multiplex PCR with that of conventional culture method suggests that the multiplex PCR is a reliable and useful method for rapid detection of *Salmonella* spp., *L. monocytogenes*, and *E. coli* 0157:H7 contamination in meat products. In conclusion, the multiplex PCR assay described in this study for the simultaneous detection of *Salmonella* spp., *L. monocytogenes*, and *E. coli* 0157:H7 is capable of detecting as few as 1 CFU/25 g of any of these organisms in raw meat after enrichment cultivation for 24 h.