

**SIMULTANEOUS QUANTITATION OF *ESCHERICHIA COLI* O157:H7,  
*SALMONELLA* AND *SHIGELLA* IN GROUND BEEF  
BY MULTIPLEX REAL-TIME PCR  
AND IMMUNOMAGNETIC SEPARATION**

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**ABSTRACT**

The objectives of this study were to establish a real-time multiplex polymerase chain reaction (PCR) for simultaneous quantitation of *Escherichia coli* O157:H7, *Salmonella* and *Shigella* that have been implicated in a number of foodborne disease outbreaks. Genomic DNA for the real-time PCR was extracted by the boiling method. Three sets of primers and corresponding TaqMan® probes were designed to target these three pathogens. Multiplex real-time PCR was carried out with TaqMan® Universal PCR Master Mix in an ABI Prism 7700 Sequence Detection System. Final standard curves were calculated by plotting the threshold cycle (*Ct*) value against log<sub>10</sub> CFU/ml by linear regression to analyze the results for each pathogen. With optimized conditions, the quantitative detection ranges of the real-time multiplex PCR for pure cultures were 10<sup>2</sup> to 10<sup>9</sup> CFU/ml for *E. coli* O157:H7, 10<sup>3</sup> to 10<sup>9</sup> CFU/ml for *Salmonella* and 10<sup>1</sup> to 10<sup>8</sup> CFU/ml for *Shigella*. When this established multiplex real-time PCR system was applied to ground beef samples, the lowest detection concentration of three pathogens were increased to 10<sup>5</sup> CFU/g for *E. coli* O157:H7, 10<sup>3</sup> CFU/g for *Salmonella* and 10<sup>4</sup> CFU/g for *Shigella*. Immunomagnetic separation was then used to isolate *E. coli* O157:H7 and *Salmonella* from the beef samples. The lowest detection concentrations of three

pathogens were reduced to  $10^3$  CFU/g. TaqMan® real-time PCR, combined with IMS has the potential to be a faster and more reliable method for rapid quantitation of *E. coli* O157:H7, *Salmonella* and *Shigella* in food, which will take 3 h for the whole process.