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A duplex SYBR Green I real-time quantitative PCR assay for detecting *Escherichia coli* O157:H7

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ABSTRACT. PCR and hybridization assays are widely used for the detection and identification of *Escherichia coli* serogroups and serotypes. We used these techniques for the detection of *E. coli* O157:H7, a dominant serogroup among *E. coli* strains that are considered major public health problems worldwide. We developed a quantitative PCR assay using SYBR Green I, based on the published sequences of the *rfbE* and *fliC* genes from *E. coli* O157:H7. This method detected the *E. coli* O157:H7 O somatic antigen gene and the flagellar antigen gene simultaneously, with good specificity, sensitivity, and repeatability. The sensitivity of the assay was 2.95 x 10 copies/μL, which is 10^3 times more sensitive than obtained with a conventional PCR. The intra-assay and inter-assay coefficients of variation were less than 2%. We concluded that this duplex quantitative PCR assay is adequate for the identification and quantitative analysis of *E. coli* O157:H7. This provides a new identification method for clinical diagnosis of *E. coli* O157:H7 and for food safety analysis, as well as for molecular epidemiological studies of foodborne diseases.

Key words: Duplex; SYBR Green; Quantitative PCR; *Escherichia coli* O157:H7

