Abstract: EVALUATION OF MULTIPLEX SYBR GREEN REAL-TIME PCR ASSAY FOR DETECTION OF PATHOGENIC ESCHERICHIA COLI

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Pathogenic Escherichia coli (E. coli) has been implicated in a wide range of disease causing infections. It is essential to generate a method for detecting and differentiating each pathotype of E. coli which is more quickly and efficiently by using less reagent. This study aimed to evaluate a SYBR Green multiplex real-time PCR method for detecting four types of pathogenic E. coli. Two of multiplex real-time PCR system, 6-plex and 3-plex, were set to detect six different virulence factors from ETEC, EPEC, EHEC, and EIEC and evaluate the melting curves and specificity compared to simplex method. The results showed that 3-plex rt-PCR method gave more reliable melting curves than 6-plex. The 3-plex rt-PCR also provided similar melting value (Tm) to simplex system. The results of this specificity assay supported the selection of 3-plex rt-PCR conditions for detection of pathogenic E. coli.

Keywords: Melting Curve; Multiplex Rt-PCR; Pathogenic E. coli; Specificity; SYBR Green.

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