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Simultaneous Identification of Diarrheagenic *Escherichia coli* Using Thermostabilized Multiplex PCR Formulation

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***Abstract*—**The study was aimed to develop a multiplex PCR (mPCR) coupled with the thermostabilization process for the simultaneous detection of major genes of diarrheagenic *Escherichia coli* (DEC) with internal amplification control (IAC) to avoid false-negative results. The most important virulence-associated genes of major pathotypes are targeted in the mPCR assay. The genes include *aggR, eltA, bfpA, vt2* and *ipaH* along with an IAC. Further, to enhance the application of mPCR, the master mix containing all reagents except template DNA was lyophilized with an appropriate lyoprotectant and storage stabilization studies were carried out to evaluate the performance of the lyophilized master mix. The assay was able to detect 10^3 CFU/mL for the simultaneous detection of targeted genes and 10^4 CFU/g bacterial load in spiked stool samples. The shelf-life of lyophilized master mix with a suitable lyoprotectant was found to be six months at 4 ^oC and 1.5 months at ambient temperature and could be stored at ambient temperature without a cold-chain facility. Considering the importance of DEC in the field of diagnostics, rapid, robust, user-friendly detection platforms are essential to prevent any outbreaks. The results obtained in the present study supported that it could be used as a highly reliable diagnostic tool in point-of-care laboratories and also field-based investigations. Besides, the developed assay can also cut down costs in terms of storage and transportation suggesting a potential field application format.

Keywords— Diarrheagenic *Escherichia coli*; Multiplex PCR; Thermo stabilization; *E. coli* pathotypes; lyophilization.

