A diagnostic kit for the enteroviruses Coxsackie A6 and A10

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ABSTRACT. Recently, there has been an upward trend in the occurrence of hand-foot-mouth disease, which is correlated with Coxsackie A6 and A10 infections. Although two separate diagnostic reagents are available for these two viral strains, the protocol and diagnosis efficiency still need to be improved. More importantly, as co-infection with these viruses is common, the development of a single test kit that can diagnose both viruses would be most beneficial for clinical practice. In our study, specific primers targeting viral nucleic acids were designed and modified. Viral nucleic acids were extracted from fecal or throat swab samples by ultrasonic rupture and silicon membrane purification. The consistency, specificity, and sensitivity of the tests were further optimized by adjusting the polymerase chain
reaction (PCR) conditions. The efficiency of viral nucleic acid extraction was significantly enhanced by the ultrasonic rupture and silicon membrane elution approach. Specific amplifications of both viral nucleic acids were achieved using modified primers. The optimal conditions for PCR were also determined (60°C for 30 min and 95°C for 2 min, followed by 40 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 60°C, and elongation for 50 s at 72°C). Amplified products were confirmed as viral specific nucleotides by agarose gel electrophoresis and sequencing. The minimal nucleic acid concentration required for detection was 0.2 ng/L, which was adequate to yield satisfactory specificity and consistency. This novel diagnostic method has many advantages, including rapid protocols and accurate results, and can be promoted for large-scale clinical trials.

**Keywords:** Hand-food-mouth disease; Enterovirus coxsackie A6; Enterovirus coxsackie A10; PCR