

Thesis Title	PCR Detection of <i>Vibrio cholerae</i> Serogroups O1 and O139
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ABSTRACT

Cholera is a serious epidemic disease that has killed millions of people all over the world. In the past, the cause of cholera was the bacterium named *Vibrio cholerae* strain O1, while *V. cholerae* non-O1 are associated with sporadic cases of diarrhea and extraintestinal infections. Recently, there was an emergence of cholera caused by *V. cholerae* non-O1 strain O139 synonym Bengal which is similar to *V. cholerae* O1 biotype El Tor. The clinical features of cholera produced by both strains are essentially identical. The conventional method for detection of *V. cholerae* is by culture method and further identification with O1 and O139 specific antisera. This method is time consuming, expensive and lacks the necessary sensitivity.

In this study, a sensitive and specific method to identify *V. cholerae* O1 and O139 by using the multiplex polymerase chain reaction was developed. The primers were designed from the cholera toxin and the O-antigen genes of *V. cholerae* O1. The 786 bp and 557 bp PCR products were obtained when *V. cholerae* O1 were tested, while only the 786 bp product was recovered in the case of *V. cholerae* O139. The optimum condition for multiplex PCR was 200 μ M of each dNTP, 1.5 mM MgCl₂ in PCR buffer pH 8.3, 0.05 μ M of each primer from *rfbQRS*, 0.5 μ M of each primer from *ctxAB*, and 1U of *Taq* DNA polymerase. The amplification reaction was carried out for 40 cycles, each cycle consisted of denaturation at 90°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 1 min. The sensitivity of this PCR protocol was

10 pg of purified DNA, 10^5 bacterial cells from pure culture and 2.5×10^5 cells from artificially contaminated stool. The specificity of the multiplex PCR was also examined and no cross reaction was found with other 22 gram negative bacteria. However, this method cannot distinguish *V. cholerae* O1 and O139 in mixed infection. It is suggested that this problem may be solved by designing the third set of primers of the specific gene that encodes O139 LPS or *V. cholerae* non-O1 capsule.

The multiplex PCR has also been used to detect *V. cholerae* in watery stool samples in comparison with the conventional culture method. All positive cases by PCR were *V. cholerae* O1. Comparative study on the detection of *V. cholerae* by microbiological method and multiplex PCR were performed using neat stools and Chelex[®]100 treated stools of 115 diarrheic patients as templates. It was found that the diagnostic sensitivity, specificity, efficacy, positive and negative predictive values of detection of the multiplex PCR were 64.28, 99.01, 94.78, 90.0 and 95.24%, respectively. The values were 100, 97.03, 97.39, 82.35 and 100%, respectively when APW enriched stools were used as templates. The study suggests that enriched stool with APW is the best template to detect *V. cholerae* by multiplex PCR.