Thesis Title Rapid Diagnosis for Detection of Cholera Toxin Gene by Using Digoxigenin Labeled Oligonucleotide Probe in Frozen Shrimp Samples

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ABSTRACT

The study on rapid diagnosis for detection of cholera toxin (CT) gene by using digoxigenin-labeled 23-base *ctxA* oligonucleotide probe was carried out in 111 frozen shrimp samples in Samutsakorn Province, during May 1995 to July 1996. The sensitivity of the oligonucleotide probe determined by dot blot hybridization with extracted genomic DNA of reference strain *V. cholerae* O1 AQ 1034 (CT^+) was 1 ng or 1 *ug*/ml and 10^6 colony forming unit (CFU)/ml. The specificity of *ctxA* oligonucleotide probe was determined by colony hybridization technique. All 149 strains of *V. cholerae* O1 and O139 were hybridized with the digoxigenin-labeled *ctxA* oligonucleotide probe, while a total of 240 strains of *V. cholerae* non O1 and other enteric bacteria were not hybridized. The *ctxA* oligonucleotide probe was then used for the

rapid detection of toxigenic *V. cholerae* in 111 frozen shrimp samples. All DNA samples gave negative results for *ctx*A by dot blot hybridization assay, and *V. cholerae* 01 organisms were also not detected in all samples with the microbiological method. In addition, the enteropathogens were isolated from 69 (62.2%) of 111 frozen shrimp samples. *V. parahaemolyticus* were detected in the highest frequency (39.6%), followed by *V. cholerae* non 01 (14.4%), and *Aeromonas hydrophila* (11.7%). All 368 strains of enteropathogens isolated in frozen shrimp samples were negative for *ctx*A. However, the dot blot hybridization method with digoxigenin labeled 23-base *ctx*A oligonucleotide probe was successfully developed for rapid detection of toxigenic *V. cholerae* 01 from frozen shrimp samples.