

Thesis Title Detection of *Escherichia coli* in Water and Food by using
DNA probe and Polymerase Chain Reaction.

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ABSTRACT

To develop the alternative method for detection of *Escherichia coli* in water and food, specific DNA probes and polymerase chain reaction methods for monitoring the presence of this bacteria were studied. The four digoxigenin labeled DNA probes derived from *uidA* gene of *E. coli* K12 which encodes β -glucuronidase (GUS) namely GA153, GA527, GA175, and GA549 were used in dot blot hybridization with bacterial cultures. These four probes hybridized and gave positive signal to all 120 strains of *E. coli* tested in this study independent of GUS phenotype. Other enteric bacteria except *Shigella* such as *Citrobacter*, *Enterobacter*, *Edwardsiella*, *Morganella*, *Klebsiella*, *Proteus* and *Salmonella* and other gram positive bacteria including *Bacillus* and *Staphylococcus* were not reactive with these probes. All 11 strains of *Shigella* which are both GUS positive and negative could hybridize well with the four probes. PCR amplification with two specific sets of primers derived from

uidA gene, GAL-301 with GAR-432 and GAL-301 with GAR-806 which gave product of 0.153 kb and 0.527 kb, respectively also confirmed the high specificity of detection of *E. coli* using primers from such gene. The observed data demonstrated that the use of either one of the four probes, GA153, GA527, GA175 and GA549, or the 2 pairs of the three primers, GAL-301, GAR-432 and GAL-806 proven to be highly specific for detection of *E. coli* and *Shigella* which may or may not have GUS activity. The dot blot hybridization technique, using digoxigenin labeled probes could detect *E. coli* at 10^5 CFU/dot and the sensitivity could be brought down to detect only 1 to 10 bacteria per reaction if the PCR technique was used. Detection of *E. coli* in water sample could be done using PCR amplification together with filtration technique with FHLP membrane (Millipore Corp.). The detection of *E. coli* in 10 brands of drinking water was performed by such combined technique. The result showed that *E. coli* was not found in all the ten brands of drinking water samples. Direct PCR amplification and PCR combined with filtration method to detect *E. coli* in food sample were tested. The preenrichment step increased the yield of PCR products which indicated the increase of bacterial cells upon incubation. However there was evidence demonstrated that PCR amplification could detect DNA (1 ng) which was contaminated in food sample even after incubation at 37°C for 0, 30 and 60 min. Such finding indicated that if the sample contaminated with nonviable cells or intact DNA, there was possibility that PCR technique will be able to detect DNA released from those cells or free DNA that was present in the sample. Therefore, it is suggested that the interpretation of bacteria detection in water or food by PCR technique samples requires specific monitoring factors.