

Rapid identification of LT⁺ *E. coli* by means of PCR and its test comparisons

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Abstract

AIM: To select a test method for specific, sensitive and rapid identification of LT⁺ *E. coli*.

METHODS: Stool samples inoculated into LB solution were cultured for 4 h at 35 °C. 10 μl boiled culture solution was taken to template. Two oligonucleotide primers were used in a polymerase chain reaction (PCR) procedure to amplify a highly conserved DNA sequence of the A subunit of the heat-labile enterotoxin. Detection of the 110 bp amplified product can be done by agarose gel electrophoresis. Thirty strains of known bacteria (LT⁺ *E. coli* (EC-129), ST⁺ *E. coli* (EC-130) and LT⁺ ST⁺ *E. coli* (EC-142), *Salmonella typhimurium*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella group C*, *Shigella sonnei*, *Enterobacter aerogenes*, *Alcaligenes sp.*, *Providencia rettgeri*, *Proteus mirabilis*, *Morganella morganii*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Klebsiella*

pneumoniae, *Citrobacter diversus*, *Enterobacter cloacae*, 12 strains of *E. coli* isolated from bile samples) and 108 diarrhea samples were detected. A total of 108 diarrhea samples were compared with LT probe hybridization, modified Eleck (M-Eleck) and ELISA simultaneously.

RESULTS: By PCR, of the 30 strains of bacteria, only LT⁺ *E. coli* and LT⁺ ST⁺ *E. coli* were positive; in 40 of the 108 diarrhea samples, 20 were positive and in the other 68 samples from infants, only five were found to be positive. Of the 25 positive samples by PCR, 23 were also found to be positive in the other 3 tests; 1 was found to be positive by M-Eleck and ELISA. Of the 83 negative samples by PCR, the same negative results were found by MEleck and ELISA, but 2 were found to be positive by LT probe hybridization. The overall coincidence rate was about 95%. Analysis of correlation showed a significant difference between PCR and other three tests ($P < 0.01$) and analysis of difference showed no significant difference ($P > 0.05$) between them. In the detection of LT⁺ *E. coli* by means of PCR, the minimum number of target bacteria required was 50 CFU. The whole test was finished in 7 h.

CONCLUSION: Detection of LT⁺ *E. coli* by PCR showed that the method is specific, sensitive and rapid.

Key words: *Escherichia coli*; Oligonucleotides; Polymerase chain reaction; Electrophoresis, agar gel; Diarrhea; feces; Enterotoxins

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