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Improved Detection of Heat-Labile Enterotoxin of Enterotoxigenic *Escherichia coli* by Using a Commercial Coagglutination Test

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***Escherichia coli* strains grown on lincomycin-supplemented Mundell agar and on blood agar were compared for their ability to produce heat-labile enterotoxin, as detected by a commercial coagglutination kit. The special agar allowed more strains to be detected, and the results were much more clear-cut.**

Enterotoxigenic *Escherichia coli* (ETEC) has long been recognized as an etiologic agent in diarrhea in developing countries and in traveler's diarrhea (8). ETEC comprises strains which can produce heat-labile toxin or heat-stable toxin or both, and all of these strains can be pathogenic in humans. For technical reasons, it has been easier to detect production of heat-labile toxin, and several methods have been developed for their diagnosis in the laboratory.

Among the first methods developed were the Y-1 mouse adrenal cell assay and the modified Elek (Biken) test (2, 4). These tests, although useful, are not practical for the routine laboratory, as they require either cell culture techniques or incubations of several days' duration. Enzyme-linked immunosorbent assay (ELISA), latex agglutination, and coagglutination methods (1, 3, 7, 9) represented significant advances in the diagnosis of heat-labile-toxin-producing *E. coli* infection. The methodology is not too difficult, and results, especially with latex agglutination and coagglutination, are rapidly obtainable. These methods, however, still required laboratories to construct their own systems, as commercial kits were not available.

Recently, a new commercial coagglutination kit for detection of heat-labile toxin of ETEC (Pharmacia Diagnostic AB, Uppsala, Sweden) was introduced into our laboratory. According to the instructions of the manufacturer, *E. coli* strains grown on blood agar could be directly tested. We wondered whether the yield could not be improved if bacterial strains were grown on medium as described by Mundell et al. (6), supplemented with 1.5% agar to make solidified plates, and to which was added lincomycin (45 μg/ml) (Sigma Chemical Co., St. Louis, Mo.), as lincomycin had been previously shown to enhance production of enterotoxin by ETEC (5).

In order to test this hypothesis, 300 strains of *E. coli* from 300 patients with acute gastroenteritis were tested for heat-labile enterotoxin production after inocula obtained from a single colony were simultaneously plated on blood agar and on lincomycin-supplemented Mundell media (LSMM). In addition, 55 strains previously shown by ELISA to produce heat-labile enterotoxin were retested by ELISA and by coagglutination after having been plated simultaneously on blood agar and LSMM. All of the 55 strains retested positive by ELISA and of these, 51 which were tested by cell culture assay were found to produce heat-labile enterotoxin.

The ELISA was performed by the method of Svennerholm and Wiklund (10), with ganglioside GMI (purchased from Supelco Inc., Supelco Park, Bellefonte, Pa.), rabbit anti-cholera toxin antisera (kindly provided by M. M. Levine, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore), and phosphatase-labeled affinity-purified antibody to rabbit immunoglobulin G (goat) (purchased from Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md.). The coagglutination test was performed according to the instructions of the manufacturer. A loopful of bacteria shown to be *E. coli* was suspended in extraction solution. After incubation and centrifugation, a drop of the supernatant was tested with the coagglutination-positive reagent and with the control reagent. A positive reaction is one showing stronger agglutination with the positive reagent than with the control reagent.

Of 300 strains grown on blood agar, 11 (3.7%) were identified as heat-labile-toxin-producing *E. coli*, while 23 of 300 (7.7%) strains grown on LSMM were identified as such, an increase in yield of over 100% (Table 1). All 11 strains positive on blood agar were also positive on LSMM, and all positive strains were subsequently confirmed as heat-labile-toxin-producing *E. coli* by ELISA. The results in Table 1 also show that of 55 strains shown to be heat-labile-toxin-producing *E. coli* by ELISA, 52 (94.5%) could be detected by the coagglutination method when the strains were grown on LSMM, while only 27 (49%) could be detected when the strains were grown on blood agar. In addition to the lower sensitivity obtained by use of blood agar, agglutination of positive strains grown on blood agar was much weaker than that of positive strains grown on LSMM. On the basis of these results, we strongly recommend culturing all *E. coli* strains on LSMM prior to testing them for ability to produce heat-labile enterotoxin with this commercial kit.

TABLE 1. Detection of heat-labile enterotoxin produced by *E. coli* grown on different media

Medium	No. of positive strains	
	From clinical samples (n = 300)	By ELISA (n = 55)
Blood agar	11[a]	27[b]
LSMM	23[a]	52[b]

[a] $P < 0.05$ by the Z test.
[b] $P < 0.001$ by the Z test.

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