Evaluation of Three Simple and Rapid Immunological Tests for Detection of Heat-Labile Enterotoxin of Enterotoxigenic *Escherichia coli*

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Three simple immunological tests, the modified Elek (Biken) test, the modified staphylococcal coagglutination test, and the rapid GM1-horseradish peroxidase-enzyme-linked immunosorbent assay have been evaluated for detection of heat-labile enterotoxin of enterotoxigenic *Escherichia coli*. Of the 100 coded *E. coli* strains tested, 94 gave consistent results with all the three immunological tests; a discrepancy was observed in only 6 strains. Identical results were obtained when the Biken test was conducted with complete and incomplete Biken kits (Meguro Institute Ltd., Osaka, Japan). All three immunological tests evaluated in this study were found to be sensitive and simple and can be easily adopted by any laboratory for detection of heat-labile enterotoxins of enterotoxigenic *E. coli* strains.

Enterotoxigenic *Escherichia coli* (ETEC) has been recognized as one of the major etiological agents of acute diarrheal diseases. The heat-labile toxin (LT) of ETEC isolates can be assayed in animal models, including the adult rabbit ileal loop (13) and skin permeability factor test (5), and in tissue culture systems such as Chinese hamster ovary (CHO) cells (8) and mouse Y1 adrenal cells (3). In addition to these techniques, a number of sensitive immunological methods such as solid-phase radioimmunoassay (7), the passive immune hemolysis (4), the reversed passive hemagglutination test (12), the staphylococcal coagglutination test (2), and enzyme-linked immunosorbent assay (ELISA) (19) have also been developed for detection of LT production. Animals are expensive, and handling a large number of them for LT assay is tedious and cumbersome and thus may not be suitable for routine laboratory use. Although the cell culture methods and most of the immunological assays are very sensitive, it may not be practical to undertake such tests owing to the need for expensive laboratory equipment and reagents and the lack of trained laboratory personnel.

Three simple and rapid immunological methods, the modified GM1-horseradish peroxidase-ELISA (GM1-HRP-ELISA) (18), the modified Elek (Biken) test (11), and the modified staphylococcal coagglutination test (14) (Coa test) have been developed recently for LT assay.

The present study was undertaken to evaluate the sensitivity of the three above-mentioned simplified tests. Part of the study was conducted in July 1982, when our laboratory participated in the World Health Organization interlaboratory trial to evaluate the sensitivity of the modified Elek (Biken) test, using the complete and the incomplete (vide infra) Biken kits.

**MATERIALS AND METHODS**

**Bacterial strains.** The 100 coded *E. coli* strains used in this study were received through the World Health Organization from Y. Takeda in connection with a multinational multilaboratory evaluation study carried out under the Diarrhoeal Disease Control Programme of the World Health Organization (16). A total of 50 of the strains produced LT as determined by Takeda and Honda on the basis of standard assays, including CHO cell assay and the passive immune hemolysis test (6). The strains were maintained at room temperature in Dorsset egg medium. All of the isolates were tested three times for LT production by the three immunological methods evaluated in this study.

**Detection of LT-producing ETEC by the modified Elek (Biken) test.** All of the strains were tested for LT production by the modified Elek (Biken) test as advocated previously (9–11), using the complete and incomplete Biken test kits (Meguro Institute Ltd., Osaka, Japan). The complete kit contained Biken agar no. 2 with lincomycin solution (2.7 mg/ml), polymyxin B discs (500 IU per disc), and rabbit anti-LT serum. In the incomplete kit, however, Biken agar no. 2 was not supplied and instead was prepared as recommended in our laboratory with locally available ingredients (2% Casamino Acids [Difco Laboratories, Detroit, Mich.]; 1% yeast extract [Difco]; 0.25% NaCl; 1.5% K2HPO4; 0.5% glucose; 0.05% [vol/vol] trace salt solution consisting of 5% MgSO4, 0.5% FeCl3, 2% CoCl2, 6H2O; 1.5% Noble agar [Difco] or 1.5% agarose (Loba-Chemie) [pH 7.5]). Both the complete and incomplete Biken kits were kindly provided by the World Health Organization, Geneva.

**Detection of LT-producing ETEC by modified rapid GM1-HRP-ELISA.** The reagents and antiseras for conducting the GM1-HRP-ELISA were kindly supplied by Ann-Mari Svennerholm and J. Holmgren of the Department of Medical Microbiology, University of Goteborg, Sweden. The methodology used was that recommended previously (18).

**Detection of LT-producing ETEC by modified staphylococcal Coa test.** The reagents and methodology for conducting the modified staphylococcal Coa test (14) were kindly supplied by T. Wadstrom of the Swedish University of Agricultural Sciences, Uppsala, Sweden. Briefly, a loopful of the test strain grown overnight at 37°C on 5% sheep blood agar was suspended in 0.1 ml of saline (0.85%) containing polymyxin B (2 mg/ml) and incubated for 30 min at 37°C in a water bath. Then, 0.01 ml of detergent (Triton X-100, final concentration, 0.1%) was added and further incubated for 20 min in a water bath at 37°C to obtain cell lysates. The lysates were then centrifuged (3,000 × g, 20 min, 20°C). Equal volumes (20 μl) of cell lysate and Coa test reagents were
mixed on Phadebact cellulose paper and observed for agglutination.

RESULTS

The results of our study are presented in Table 1. Of the 100 E. coli strains tested for LT activity, 94 gave consistent results with all of the three immunological tests evaluated in this study. In four of the six strains giving discrepant results, LT was detected by the modified Elek (Biken) test and GM1-HRP-ELISA but not by the Coa test; 1 strain was found to be an LT producer by the modified Elek (Biken) test and the Coa test only, and in the remaining strain, LT was detected only by the Coa test. All of the 6 discrepant strains were labeled as LT producers on the basis of several standard techniques mentioned earlier (6). Identical results were obtained when the modified Elek (Biken) test was conducted with the incomplete Biken kit. We further observed that test results could be read after 72 h when 1.5% Noble agar in the preparation of Biken agar no. 2 medium.

DISCUSSION

A number of sensitive toxicological and immunological tests have been developed in recent years for detection of LT of ETEC strains. Unfortunately, most of them are not suitable for use in poorly equipped laboratories, particularly in developing countries, or in the field. All of the three immunological tests evaluated in this study are ideal for such use because they yield quick, reproducible, and accurate results without expensive laboratory equipment. However, some of the reagents, such as LT and anti-LT serum, are still not commercially available.

The modified Elek (Biken) test is a simple gel diffusion technique and can be conducted without expensive laboratory equipment and reagents. Another advantage of this test is that the test strains can simultaneously be assayed for heat-stable toxin in 1 to 4 day-old suckling mice when three agar pieces (7 mm in diameter) are punched out from the outer periphery of the growth on Biken agar no. 2 and extracted in phosphate-buffered saline (0.01 M, pH 7.0) (10). The only drawback of the method is that test results are obtained after 96 h. However, use of 1.5% agarose in place of Noble agar may reduce this period to 72 h. The observations of our study reiterate the high degree of sensitivity of the modified Elek (Biken) test, using either the complete or the incomplete Biken kit. The incomplete Biken kit could be more convenient for laboratories in developing countries as it costs about U.S $0.50 per test in comparison to the complete Biken kit which costs about U.S. $4.00 (Meguro Institute Ltd.).

The modified staphylococcal Coa test was found to be the best among the three immunological methods in terms of simplicity. A large number of E. coli isolates could be screened for LT production within a short time, using ordinary inexpensive laboratory equipment and reagents. The reagents have been claimed to be very stable at 4°C and at room temperature (14), thus being useful for LT detection in the field.

The GM1-HRP-ELISA, which is a recent modification of a technique developed earlier (1, 15, 17, 19), is a simple and sensitive test for detection of LT-producing ETEC, and the test results can be obtained within the same working day (8 h). Other advantages of this method include simultaneous screening of a large number of E. coli isolates for LT production, stability of the reagents, and visual reading of the test results.

In conclusion we found that all three immunological tests evaluated in this study are sensitive and simple and can be easily adopted by any laboratory in a developing country provided that the reagents are readily available.

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LITERATURE CITED


EVALUATION OF TESTS FOR DETECTION OF E. COLI LT


