

## Evaluation of a Reversed Passive Latex Agglutination Test for Detection of *Escherichia coli* Heat-Labile Toxin in Culture Supernatants

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**One hundred strains of *Escherichia coli* were tested for the production of the heat-labile enterotoxin by the Y1 adrenal cell test and a commercially available reversed passive latex agglutination test. The strains were grown in Casamino Acids-yeast extract broth, and filtered culture supernatants were tested for the presence of heat-labile enterotoxin. There was perfect correlation between the Y1 test and the reversed passive latex agglutination test, and the latter was simple to perform and completed within 48 h.**

Enterotoxigenic *Escherichia coli* produces either a heat-stable enterotoxin (ST), a heat-labile enterotoxin (LT), or both toxins. Enterotoxigenic *E. coli* is an important cause of infant diarrhea in developing countries, particularly in the tropics, and in persons traveling to these countries (6, 11). Until recently, the most commonly used tests for free LT in culture filtrates or fecal specimens have been tissue culture tests, such as the Y1 mouse adrenal cell test and the Chinese hamster ovary cell test. These are not suitable for use in diagnostic laboratories.

Several groups have described tests for LT using immunological assays such as enzyme-linked immunoassays and passive hemagglutination (1, 3, 15). However, the reagents for these tests have not been available for general use. In the Biken test, LT is detected by the appearance of a precipitin line between growth of the organism and antiserum against LT (8, 9); reagents for this test are available commercially. It is easily performed and requires little equipment, but it takes 5 days for the test to be completed (14). Another immunological assay which has been described is a latex bead agglutination test (4, 5, 10); this is an easy technique which uses stable reagents. A kit for LT detection using a reversed passive latex agglutination (RPLA) test is now available commercially. This paper describes the evaluation of this test kit (VET-RPLA; Oxoid Ltd., Basingstoke, England) for the detection of LT production by strains of *E. coli*.

A total of 100 strains of *E. coli* was tested. This collection of strains was used several years ago for a World Health Organization-sponsored evaluation of the Biken test (14). The strains were isolated at the Osaka (Japan) International Airport from patients who had just returned from Southeast Asian countries. The strains were received in the Division of Enteric Pathogens on Dorset egg agar slopes from Y. Takeda in 1981 and stored at room temperature. They included nine replicate cultures of one LT-producing (LT<sup>+</sup>) strain, 536-2, and nine replicate cultures of one nontoxicogenic strain, 8-669-1. Since the production of LT is known to be plasmid encoded, it may not be a completely stable character in some strains. Therefore, for this study single colonies from each strain were retested for LT production by the Y1 cell test described below and for ST production by the method of Dean et al. (2). When possible, a colony with the toxigenic characteristics of the original culture was selected. It was

not possible to reisolate colonies of seven of the strains with the same toxin-producing ability as reported for the original culture in the earlier Biken test evaluation. Replacement cultures of these strains were not available from Y. Takeda. Five of these strains were originally ST producing (ST<sup>+</sup>) but were now nontoxicogenic. Two of the strains had originally produced ST and LT but now produced ST only. The 100 strains were characterized as follows: 19 were ST<sup>+</sup> LT<sup>+</sup>, 31 were ST<sup>-</sup> LT<sup>+</sup>, 16 were ST<sup>+</sup> LT<sup>-</sup>, and 34 were ST<sup>-</sup> LT<sup>-</sup>. Cultures of the characterized single colonies were preserved by freeze-drying.

The method recommended in the leaflet of the manufacturer was used for the preparation of culture filtrates for testing with the VET-RPLA kit. The same preparations were used for the Y1 cell test. The strains to be tested were grown in broth containing 2.0% Casamino Acids (Difco Laboratories), 0.6% yeast extract, 0.25% NaCl, 0.87% K<sub>2</sub>HPO<sub>4</sub>, and 0.1% (vol/vol) trace salts solution and adjusted to a pH of 8.5. The trace salts solution contained 5% MgSO<sub>4</sub>, 0.5% MnCl<sub>2</sub>, and 0.5% FeCl<sub>3</sub> dissolved in 0.001 N H<sub>2</sub>SO<sub>4</sub>. A 10-ml sample of broth in a 250-ml flask was inoculated and incubated with shaking at 37°C for 24 h (at approximately 130 oscillations per min). After centrifugation, the culture supernatants were filtered through disposable Acrodisc filters (pore size, 0.45 μm; Gelman Sciences, Inc.).

Doubling dilutions of filtered supernatants were tested for LT production by the Y1 cell test (12). The titer was the highest dilution causing rounding of at least 50% of Y1 cells. LT was detected in the culture filtrates of 50 strains (see Table 1). The titers in the Y1 test ranged from 10 to 2,560, and 36 LT<sup>+</sup> strains gave titers equal to or greater than 320.

The VET-RPLA test kit included a reference control of lyophilized *Vibrio cholerae* enterotoxin (cholera toxin), a latex suspension sensitized with antibodies to cholera toxin, a control latex suspension coated with nonimmune rabbit globulins, and a diluent of phosphate-buffered saline containing 0.5% bovine serum albumin and 0.1% sodium azide. The VET-RPLA test was performed in a V-type microdilution plate (Sterilin). Doubling dilutions of the test supernatants were made in 25 μl of the supplied diluent across seven of eight wells by using a digital multichannel pipette. Diluent alone (25 μl) was placed in well 8. Two rows were prepared for each sample. Sensitized latex suspension (25 μl) was added to each well of row 1, and 25 μl of control latex suspension was added to each well of row 2. Therefore,

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TABLE 1. VET-RPLA test for detection of LT

VET-RPLA test titer	No. of strains producing LT <sup>a</sup> (n = 50)
2	1
4	8
8	6
16	5
32	11
64	11
128	8

<sup>a</sup> By Y1 cell test.

samples were tested at dilutions ranging from 2 to 128. The contents of the wells were mixed by using a microplate mixer (Dynatech Laboratories, Inc.) for 30 s. The plate was covered with plastic plate sealer to prevent evaporation and left for 24 h at room temperature. The results were read against a dark background. A result was negative when there was a pinpoint pellet similar to that seen in the row containing unsensitized latex beads. Turbid zones taking up 100, 75, and 50% of the well were scored as +++, ++, and +, respectively. The titer was the highest dilution giving a reading of at least +. All tests with the unsensitized control beads were negative, as were the diluent controls. All 50 strains negative in the Y1 cell test were negative with sensitized beads. All 50 strains positive in the Y1 cell test were positive in the VET-RPLA test, with titers ranging from 2 to 128; 8 of the 50 gave the maximum titer of 128 (Table 1). The cholera toxin control preparation gave a titer of 32 with sensitized beads and did not react with the control beads.

Some strains of *E. coli* (7) produce a toxin (LT-II) which is active on Y1 cells but which is immunologically distinguishable from the LT described in this paper, which has been termed LT-I. LT-II is not neutralized by antiserum against LT-I or cholera toxin and is not detected in immunological tests, such as enzyme immunoassays, that use these sera. We tested the three strains in our culture collection which are known to produce LT-II, and although they were readily detected by using the Y1 test they were not detected by using the VET-RPLA test. There have been few studies to determine the prevalence of *E. coli* strains producing LT-II; these organisms were rarely isolated from humans and were isolated more frequently from cattle (13).

By using the VET-RPLA test, the presence of LT-I in the culture supernatants of 100 strains of *E. coli* was determined correctly as judged by the simultaneous testing of the supernatants in a Y1 cell test. The VET-RPLA test can be recommended even though it requires the use of flasks, filters, and a rotary shaker, because it is easy to perform and after the preparation of the filtrates the result is available in 1 day. It is therefore considerably faster than the Biken test. Results are also available after 1 day when the Y1 test is used, but the VET-RPLA test has the advantage that it does

not require the expensive maintenance of a tissue culture line.

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