Rapid Latex Particle Agglutination Test for *Escherichia coli* Strains of Porcine Origin Producing Heat-Labile Enterotoxin

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A latex particle agglutination test previously shown to be suitable for the rapid identification of *Escherichia coli* strains of human origin producing heat-labile enterotoxin (R. A. Finkelstein and Z. Yang, J. Clin. Microbiol. 18:23-28) is equally applicable to strains of porcine origin.

Despite the importance of the problem, until recently it has been difficult and generally impractical to identify strains of *Escherichia coli* which elaborate diarrheagenic enterotoxins. The introduction of the Biken test (8) was a significant development in that it is simple, reliable, and economical. However, it takes 3 to 4 days to obtain results. The use of gene-specific DNA probes (12) provides the distinct advantage of being directly applicable to stool samples, obviating the need for culture, but at the present time, this requires a radiolabeled, cloned gene probe and several days to develop autoradiograms (although faster procedures are being developed).

We have recently introduced (5) a latex particle agglutination test (LPAT) which is suitable for the rapid recognition of heat-labile enterotoxin (LT)-producing colonies of *E. coli*. The test is simple and economical to perform; it is directly applicable to colonies on a variety of commonly used enteric diagnostic isolation media; and the results are available in minutes (5). However, the previous results were obtained only with a series of enterotoxigenic and non-enterotoxigenic strains of human origin. As the LPAT is an immunologically based test and it is known (6, 10, 11) that, despite their overall structural, functional, and immunological similarity, there are significant differences between LT from strains of human origin (H-LT) and LT from strains of porcine origin (P-LT), the purpose of this study was to determine whether the LPAT was suitable for the detection of P-LT-producing strains of *E. coli*. To this end, a

comparison was made of 106 porcine *E. coli* isolates of diverse origin by the Y-1 adrenal cell assay (4), the Biken test as modified (5), the DNA probe technique (12), and the LPAT (5), using affinity-purified polyclonal goat antibody against H-LT (6) and against P-LT (2).

The *E. coli* strains, from the collection at the National Animal Disease Center, included diverse serotypes (O, K, H, and nonmotile) from the United States, Switzerland, England, Hungary, Sweden, Canada, and Panama. Forty-five strains produced LT as demonstrated with tryptic soy broth culture supernatants in the Y-1 adrenal cell assay (4). Forty-six strains, including 10 of the LT-positive strains, produced heat-stable enterotoxin (ST) (25 ST_a, 18 ST_b, and 3 ST_a and ST_b) as determined by the infant mouse assay for ST_a (7) and the pig intestinal loop assay for ST_b (1). The latter observations, which are irrelevant to the present study, are mentioned to illustrate the diversity of the strains tested. The Y-1 adrenal cell assays, the DNA probe technique, and the tests for ST were performed in the National Animal Disease Center on all of the strains which were sent (as serially numbered slants without other identifying data) to the University of Missouri laboratory for the Biken test and LPAT determinations. After these results were obtained, the data were compared.

All but 1 of the 45 strains which produced LT demonstrable in the Y-1 adrenal cell assay were also positive by the DNA probe technique (12) and the modified Biken test (5) and in the LPAT performed as described previously (5), using colonies on MacConkey agar and latex sensitized with either immunoaffinity-purified anti-H-LT or immunopurified anti-P-LT. (The single exception was a strain which was negative in the Biken test only.) Conversely, the 61 strains which lacked activity in the Y-1 adrenal cell

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assay were negative in all of the other assays as well. Thus, there was virtually 100% concordance of the results of all assays with these strains.

The finding that the LPAT which was developed with purified anti-H-LT antibody was equally as suitable as the LPAT with purified anti-P-LT in recognizing P-LT-producing *E. coli* strains was somewhat surprising because the test is ca. 3 to 4 times more sensitive when homologous reagents are used. For example, the LPAT with purified anti-H-LT can detect pure H-LT at 0.03 µg/ml whereas ca. 0.08 µg of pure P-LT per ml is required for a positive reaction. Thus, it was anticipated that separate tests might be necessary for porcine and human strains. Apparently, however, the margin of sensitivity is sufficient to enable the anti-H-LT LPAT to detect P-LT-producing strains. The reverse (i.e., the use of anti-P-LT to recognize H-LT-producing strains) has not been tested. It should also be mentioned that during the present study we found that the addition of Na₂S₂O₃, 1% final concentration, to the sensitized latex preparations did not affect the sensitivity or specificity of the test but increased the shelf life of the sensitized latex even further (5). Sensitized latex, preserved with azide and maintained in the liquid state at ambient laboratory temperature (22 to 23°C), retained its suitability and sensitivity for at least 3 months, the longest period tested.

Other rapid tests for identification of LT-producing *E. coli* have been introduced while this work was in progress. A staphylococcal coagglutination test, thus far evaluated only with human *E. coli* strains (9), requires an additional subculture period of 5 to 6 h after primary culture. A variation of that test (13) was more rapid, but gave negative results with 4 of 43 LT⁺ human strains and one-third of the LT⁺ animal strains tested. Paradoxically, the latter authors indicated that cells harvested from MacConkey agar were not suitable. A modified enzyme-linked immunosorbent assay (3) requires additional overnight subculture onto special agar plates coated with GM1 ganglioside and gives nonspecific reactions unless particular monoclonal antibodies and corresponding rabbit anti-mouse immunoglobulin conjugate are used.

We conclude that the LPAT which we have described has advantages over each of the other assays. It is simple, economical, and quick; it is applicable to the primary colony on a variety of commonly used enteric isolation media without

need for subculture; and it is suitable for detecting both H-LT- and P-LT-producing isolates.

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