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Evaluation and Optimization of a Latex Agglutination Assay for Detection of Cholera Toxin and *Escherichia coli* Heat-Labile Toxin

WING CHEONG YAM,* MARIA LI LUNG,† AND MUN HON NG

Department of Microbiology, University of Hong Kong, Hong Kong

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The effectiveness of a latex agglutination assay kit for the detection of *Escherichia coli* heat-labile toxin and cholera toxin was determined for the identification of natural isolates of the corresponding enteric pathogens in Southeast Asia. By selection of the appropriate culture media, the sensitivity of the assay was improved from 90.6% (for the detection of heat-labile toxin) and 75% (for the detection of cholera toxin) to 100%, and the results were confirmed with bioassays and DNA hybridization assays for both clinical and environmental isolates.

Enterotoxigenic *Escherichia coli* (ETEC) and *Vibrio cholerae* cause diarrhea by releasing enterotoxins which interact with intestinal epithelial cells, causing them to secrete water and electrolytes. ETEC is a common cause of childhood diarrheas in developing countries (6). Cholera is pandemic in many regions of Asia, Africa, India, and South America (4, 14). It occurs sporadically or as confined outbreaks in other parts of the world. The disease is caused by toxigenic strains of *V. cholerae*, the principle pathogenic attribute of which is due to an enterotoxin, cholera toxin (CT). CT is chromosomally mediated and shares extensive DNA sequence homology with the plasmid-borne heat-labile enterotoxin (LT) gene of ETEC (10). It was shown previously that both pathogens could be reliably identified by using a DNA filter assay with the LT gene as the probe (15–18). Nevertheless, immunoassays are more convenient for routine applications and are especially useful where laboratory facilities are lacking. Immunological methods such as enzyme-linked immunoassays and immunoagglutination assays have been described for the detection of the heat-labile toxin produced by ETEC and the cholera toxin produced by *V. cholerae* (2, 8, 11, 13). Since antisera raised against CT cross-react with LT, a new diagnostic kit comprising latex particles sensitized with antiserum against CT was recently made available commercially for the detection of these enteropathogenic bacteria by the respective toxins they elaborate. This reversed passive latex agglutination kit does not require elaborate laboratory facilities and hence is expected to be especially useful in the above-mentioned areas of the world, where these diseases are common. The present study was undertaken in order to evaluate the reagent by testing it against large collections of ETEC and *V. cholerae* isolated from different regions in Southeast Asia. We showed that by using the appropriate culture media, this latex agglutination kit can afford reliable identification of these enteric pathogens.

This diagnostic kit was evaluated against 131 isolates of *E. coli* and 74 isolates of *V. cholerae*. As described previously (17, 18), all *E. coli* isolates were clinical isolates collected between 1983 and 1985 from Hong Kong, Japan, and the People's Republic of China. Fifty-three of them harbor the LT gene, and the remaining 78 strains harbor one or more of the other enterotoxin genes but not the LT gene. In this study, isolated colonies from each of these strains were tested simultaneously by using the DNA filter assay described previously (17) and a latex agglutination assay. In the latter instance, bacterial colonies were grown in Mundell's medium as recommended by the manufacturer and Biken's broth containing lincomycin at a concentration of 90 μg/ml (7). A 10-ml sample of each broth in a 250-ml flask was inoculated and incubated at 37°C with shaking at 130 rpm. To the overnight Mundell's medium and 40-h Biken's medium, polymyxin B was added to a concentration of 10,000 IU/ml, and then cultures were incubated further for another 4 h with shaking. Incubated broth cultures were subjected to centrifugation at 900 × *g* for 20 min at 4°C. One milliliter of the supernatant was recentrifuged in a 1.5-ml microcentrifuge tube at 8,000 × *g* for 15 min at 4°C. Dilutions of the test supernatants were made in duplicate in 25 μl of the supplied diluent in V-type microtiter plates. Samples were tested at twofold dilutions from 1 to 64. The last well contained diluent only. Two rows were prepared for each sample. Latex suspensions sensitized with antibodies to CT were added in 25-μl volumes to each well of row 1, and 25 μl of control latex suspension was added to each well of row 2. Reference CT was supplied as a positive control for each experiment. Plates were agitated carefully, covered with a lid, and left undisturbed at room temperature for 20 to 24 h. Each well was examined macroscopically for agglutination, and the titer was recorded as the reciprocal of the maximum dilution which caused agglutination of the anti-CT-sensitized latex particles.

We similarly evaluated the latex agglutination assay with 56 clinical isolates of toxigenic *V. cholerae* El Tor collected from Hong Kong, the People's Republic of China, Vietnam, Nepal, Thailand, and the Philippines (15, 16). We also tested 18 *V. cholerae* non-O1 strains isolated from 3 clinical and 15 environmental samples from Hong Kong. All of the *V. cholerae* isolates were tested for active toxin production by using the rabbit ileal loop assay as described elsewhere (3). For the latex agglutination test, *V. cholerae* strains were incubated in peptone water at 30°C for 24 h with shaking. Modified Kausama and Craig's medium (1) was also employed for a parallel study in order to evaluate the efficacies

* Corresponding author.
† Present address: Department of Biology, Hong Kong University of Science and Technology, Hong Kong.

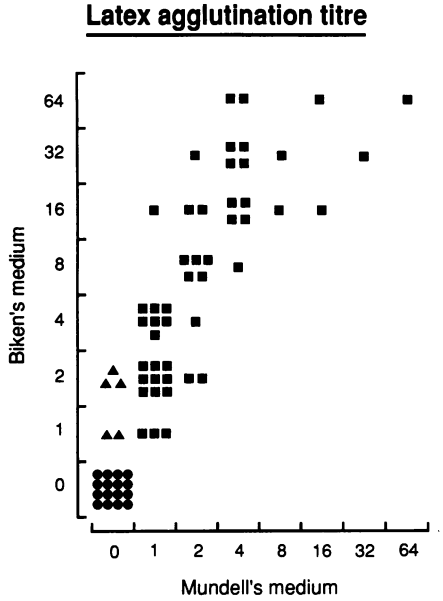

FIG. 1. Comparison of latex agglutination titers of toxigenic *E. coli* isolates cultured in Mundell's medium and Biken's medium. Seventy-eight strains (●) showed negative results with both media, 5 strains (▲) showed positive results with Biken's medium but negative results with Mundell's medium, and 48 strains (■) showed positive results with both media.

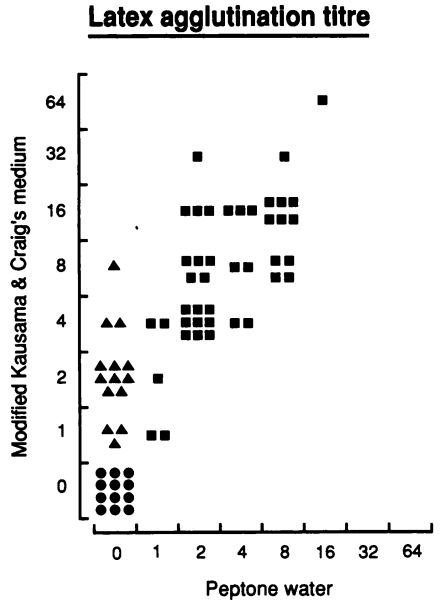

FIG. 2. Comparison of latex agglutination titers of *V. cholerae* cultured in peptone water with those cultured in modified Kausama and Craig's medium. Eighteen strains (●) showed negative results with both media, 14 strains (▲) showed positive results with modified Kausama and Craig's medium but negative results with peptone water, and 42 strains (■) showed positive results with both media.

of toxin production in these different media. Cultures were grown in 5-ml volumes in screw-cap tubes (16 by 125 mm) without shaking at 30°C for 24 h. Supernatants were titrated for elaborated toxin as described for ETEC.

Figure 1 compares the titers of LT produced by individual isolates of ETEC in Mundell's medium with those produced in Biken's medium. Seventy-eight isolates which did not harbor the LT gene gave a negative result by the DNA filter assay. These non-LT-producing ETEC isolates also did not elaborate detectable LT in either of the media. The other 53 isolates were confirmed by the DNA filter assay to be harboring the LT gene, but the titers of toxin produced varied among the isolates by as much as 64-fold. By using the paired *t* test, we found that there was a significant difference in the latex agglutination titers of Mundell's medium and Biken's medium ($P < 0.001$), and that under no circumstances was the latex agglutination titer attained from Mundell's medium higher than that attained from Biken's medium for the same isolate. The increased sensitivity was especially important for the five strains that were detected only in Biken's medium and not in Mundell's medium used as recommended by the manufacturer. With Biken's medium, the sensitivity of the assay was increased from 90.6 to 100%.

All of the 18 nontoxigenic *V. cholerae* non-O1 strains showed negative results with the latex agglutination assay and the rabbit ileal loop assay. Figure 2 compares the titers of CT produced in peptone water with those produced in modified Kausama and Craig's medium. The amounts of toxin produced varied among the remaining 56 isolates which were positive by the rabbit ileal loop assay and previous DNA filter assay (15, 16). Significantly, all of the toxigenic isolates gave a higher titer in modified Kausama and Craig's medium than in peptone water used as recommended by the manufacturer ($P < 0.001$). The increased sensitivity was further evidenced by the 14 isolates which failed to elaborate detectable toxin in peptone water but yielded a latex agglutination titer of up to 8 in modified Kausama and Craig's medium. The other 42 isolates produced detectable amounts of CT in both media. With modified Kausama and Craig's medium, the sensitivity of the assay was increased from 75 to 100%.

The ETEC and *V. cholerae* strains included in this study are representative of an extensive region in which diseases caused by these organisms are endemic. For *V. cholerae*, the sensitivity and the specificity of the latex agglutination assay obtained in this study were comparable to previous findings (1). It is evident that extracellular toxin production from our collection of *V. cholerae* El Tor isolates propagated in peptone water could not be detected by the latex agglutination assay, although the commercial kit was able to detect 1 to 2 ng of CT per ml in the broth cultures of standard strains. Modified Kausama and Craig's medium was better able than peptone water to support CT production by *V. cholerae* El Tor isolates but not that by classical vibrios (5). The use of the former also eliminates the need for shaking, the lack of which would probably be preferred by most laboratories in developing countries. Natural isolates of *V. cholerae* harbor a variable number of copies of the CT gene; some of them harbor only one copy of the gene per cell. This may account for the minor disagreement between the results obtained previously (1). However, no such discrepancy was encountered in this study, although all of our *V. cholerae* isolates harbor a single copy of the CT gene (15, 16).

For ETEC, this assay was found to be less sensitive with Mundell's medium than was reported in a previous study (12). Since this latex agglutination assay and Biken's test rely on antigen-antibody reactions, Biken's medium has been found to be superior to Mundell's medium for LT

production (7). The incorporation of lincomycin in Biken's medium enhances the detection of these organisms (9). The addition of polymyxin B after bacterial growth also increases the toxin released into the medium. This would probably account for better detection of LT than of CT with this latex agglutination kit. The only disadvantage of using lincomycin is that *E. coli* isolates require 2 days of incubation before they attain a turbidity suitable for the test.

It is understood that manufacturers design generalized media for the convenience of the user, but the efficacy of the culture medium for toxin production is a crucial determinant of the sensitivity of the test kit. For *V. cholerae*, users should be reminded to use a medium suitable for the different biotypes of *V. cholerae* O1, as we have used modified Kausama and Craig's medium for our *V. cholerae* El Tor isolates. In this test, ETEC is detected by using the cross-reacting anti-CT. It seems appropriate under such circumstances to include a standard low-toxin producer of ETEC as a control. Such simple modifications of existing kits can enhance the sensitivities of the latex agglutination assays for the detection of both enteric pathogens.

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REFERENCES

1. **Almeida, R. J., F. W. Hickman-Brenner, E. G. Sowers, N. D. Puhr, J. J. Farmer III, and I. K. Wachsmuth.** 1990. Comparison of a latex agglutination assay and an enzyme-linked immunosorbent assay for detecting cholera toxin. J. Clin. Microbiol. **28:**128–130.
2. **Beutin, L., L. Bode, T. Richter, G. Peltre, and R. Stephen.** 1984. Rapid visual detection of *Escherichia coli* and *Vibrio cholerae* heat-labile enterotoxins by nitrocellulose enzyme-linked immunosorbent assay. J. Clin. Microbiol. **19:**371–375.
3. **Burrows, W., and G. M. Musteikis.** 1966. Cholera infection and toxin in the rabbit ileal loop. J. Infect. Dis. **116:**183–190.
4. **Centers for Disease Control.** 1991. Update: cholera outbreak—Peru, Ecuador, and Colombia. Morbid. Mortal. Weekly Rep. **40:**225–227.
5. **Craig, J. P.** 1985. The vibrio diseases in 1982: an overview, p. 11–23. *In* Y. Takeda and T. Miwatani (ed.), Bacterial diarrheal disease. KTK Scientific Publishers, Tokyo.
6. **Gross, R. J., and B. Rowe.** 1985. *Escherichia coli* diarrhoea. J. Hyg. Camb. **95:**531–550.
7. **Honda, T., S. Taga, Y. Takeda, and T. Miwatani.** 1981. Modified Elek test for detection of heat-labile enterotoxin of enterotoxigenic *Escherichia coli*. J. Clin. Microbiol. **13:**1–5.
8. **Ito, T., S. Kuwahara, and T. Yokota.** 1983. Automatic and manual latex agglutination tests for measurement of cholera toxin and heat-labile enterotoxin of *Escherichia coli*. J. Clin. Microbiol. **17:**7–12.
9. **Levner, M., F. P. Wiener, and B. A. Rubin.** 1977. Induction of *Escherichia coli* and *Vibrio cholerae* enterotoxin by an inhibitor of protein synthesis. Infect. Immun. **15:**132–137.
10. **Lockman, H., and J. B. Kaper.** 1983. Nucleotide sequence analysis of the A2 and B subunits of *Vibrio cholerae* enterotoxin. J. Biol. Chem. **258:**13722–13726.
11. **Rönnberg, B., and T. Wadström.** 1983. Rapid detection by a coagglutination test of heat-labile enterotoxin in cell lysates from blood agar-grown *Escherichia coli*. J. Clin. Microbiol. **17:**1021–1025.
12. **Scotland, S. M., R. H. Flomen, and B. Rowe.** 1989. Evaluation of a reversed passive latex agglutination test for detection of *Escherichia coli* heat-labile toxin in culture supernatants. J. Clin. Microbiol. **27:**339–340.
13. **Svennerholm, A.-M., and G. Wiklund.** 1983. Rapid GM1-enzyme-linked immunosorbent assay with visual reading for identification of *Escherichia coli* heat-labile enterotoxin. J. Clin. Microbiol. **17:**596–600.
14. **World Health Organization.** 1985. Recent advances in cholera research: memorandum from a WHO meeting. Bull. W.H.O. **63:**841–848.
15. **Yam, W. C., M. L. Lung, K. Y. Ng, and M. H. Ng.** 1989. Molecular epidemiology of *Vibrio cholerae* in Hong Kong. J. Clin. Microbiol. **27:**1900–1902.
16. **Yam, W. C., M. L. Lung, and M. H. Ng.** 1991. Restriction fragment length polymorphism analysis of *Vibrio cholerae* strains associated with a cholera outbreak in Hong Kong. J. Clin. Microbiol. **29:**1058–1059.
17. **Yam, W. C., M. L. Lung, and M. H. Ng.** 1986. Evaluation and optimization of the DNA filter assay for direct detection of enterotoxigenic *Escherichia coli* in the presence of stool coliforms. J. Clin. Microbiol. **24:**149–151.
18. **Yam, W. C., M. L. Lung, C. Y. Yeung, J. S. Tam, and M. H. Ng.** 1987. *Escherichia coli* associated with childhood diarrheas. J. Clin. Microbiol. **25:**2145–2149.