JOURNAL OF CLINICAL MICROBIOLOGY, June 1994, p. 1589–1590
0095-1137/94/$04.00+0

Vol. 32, No. 6

Development and Evaluation of Rapid Monoclonal Antibody-Based Coagglutination Test for Direct Detection of *Vibrio cholerae* O139 Synonym Bengal in Stool Samples

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Received 21 December 1993/Returned for modification 10 February 1994/Accepted 10 March 1994

A monoclonal antibody-based coagglutination test directly detected *Vibrio cholerae* O139 synonym Bengal in 83 of 120 watery diarrheal stool specimens; on culture, 90 samples were positive. Thus, with 92% sensitivity, 100% specificity, and 100% positive and 95% negative predictive values, the coagglutination test is a useful rapid test for *V. cholerae* O139.

Until recently, only *Vibrio cholerae* serogroup O1 was known to be responsible for epidemics and pandemics of cholera. *V. cholerae* non-O1 serogroups were associated with sporadic cases of diarrhea and extraintestinal infections (3). However, epidemics of cholera-like disease due to *V. cholerae* non-O1 strains broke out in southern and eastern India and southern Bangladesh in the late part of 1992 (1, 6) and have since engulfed the entire Indian subcontinent. There are now documented cases of diarrhea due to this strain in many Asian countries, including Nepal, China, Thailand, and Malaysia, and Saudi Arabia. The infection due to this strain possibly heralds the beginning of the eighth pandemic of cholera (8). The strain is serologically unrelated to *V. cholerae* O1 and to the other 137 known serogroups of *V. cholerae* non-O1; therefore, it is assigned to a new serogroup, O139, with the synonym Bengal to refer to its first isolation from the coastal areas of the Bay of Bengal (7).

Laboratory diagnosis of cholera by culture takes at least 24 to 48 h, and laboratory facilities are either inadequate or nonexistent in underdeveloped parts of the world, where cholera cases usually occur. Therefore, for quick confirmation of cases and mobilization of resources for rapid treatment and containment of outbreaks, simple and rapid diagnostic tests that can be used in the field are essential. For cholera due to *V. cholerae* O1, such a test, known as Cholera Screen, has been developed (2). This is a mouse monoclonal antibody (MAb)-based coagglutination test (COAT). We have previously reported the production of highly specific MAbs to the lipopolysaccharide antigens of *V. cholerae* O139 (4). We used two of these MAbs to develop a simple and rapid COAT for the direct detection of *V. cholerae* O139 in feces. The development of the test was patterned on the Cholera Screen test for *V. cholerae* O1, which is highly sensitive and specific for detection of *V. cholerae* O1 (2).

The two MAbs used were ICL11, of the immunoglobulin G3 isotype, and ICL12, of the immunoglobulin G2b isotype. To make the test reagent, 100 μl of ascites fluid was adsorbed onto 1 ml of a 10% (vol/vol) stabilized suspension of *Staphylococcus aureus* Cowan 1 cells by a previously described procedure (5). The control reagent was prepared by adsorbing 100 μl of unimmunized mouse serum to *S. aureus* cell suspension as for the preparation of the test reagent.

The test was initially evaluated against 114 reference cultures from our culture collection (Table 1). For this purpose, pure cultures grown on Trypticase soy agar or gelatin agar plates (GIBCO, Grand Island, N.Y.) for 18 h at 37°C were suspended in physiological saline to a concentration of 10^9 organisms per ml. Both live and heated (for 2 min over a Bunsen burner or spirit lamp) suspensions were tested by a slide agglutination test. To test a sample, 2 drops were placed onto areas marked on a slide. One drop of the test reagent was added to one area, and 1 drop of control reagent was added to the other area. The slide was then gently rotated by hand for up to 2 min. The occurrence of agglutination in the test area only was considered positive; agglutination in both regions indicated indeterminate results. All 50 *V. cholerae* O139 isolates gave agglutination, and none of the other bacteria did so.

For detection of *V. cholerae* O139, single stool samples were obtained from 230 patients with cholera-like illness who were seen at the Clinical Research Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh, in Dhaka and Matlab, between August and October 1993. The stool samples were cultured for vibrios and other enteric bacterial pathogens as described previously (9). An aliquot of the stool sample was left on the bench for several minutes to allow settling of heavy fecal material, and the supernatant was filtered through a 0.65-μm-pore membrane filter (Sartorius, Göttingen, Germany), heated, and tested for coagglutination as described above.

By culture, 90 samples yielded *V. cholerae* O139; however, only 83 of these samples were positive by COAT. A COAT conducted separately with the reagents made from the two MAbs gave identical results, and no nonspecific reaction was observed. Of the 140 samples negative for *V. cholerae* O139, 20 yielded *V. cholerae* O1, 3 yielded non-O1, non-O139 *V. cholerae*, 11 yielded enterotoxigenic *Escherichia coli*, 5 yielded *Campylobacter jejuni*, and 3 yielded *Shigella* spp. on culture. None of these samples reacted in the COAT. No recognized bacterial pathogens were identified in the remaining 98 samples, and all of these samples were also negative in the COAT.

The seven samples positive by culture but negative by COAT for *V. cholerae* O139 yielded very few colonies of *V. cholerae*

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TABLE 1. Specificity of the COAT for *V. cholerae* O139

Species	No. of strains tested	% Positive
V. cholerae O139	50	100
V. cholerae O1		
Ogawa	10	0
Inaba	2	0
Salmonella spp.	4	0
Campylobacter spp.	2	0
V. cholerae other than O1 and O139	10	0
V. fluvialis	2	0
Aeromonas spp.	4	0
Shigella spp.	10	0
E. coli	10	0
Klebsiella spp.	4	0
Proteus spp.	2	0
Providencia spp.	4	0

O139 on culture plates (3 to 20 colonies). It was therefore concluded that small numbers of *V. cholerae* O139 present in stools may not be detectable by COAT. To find out the lower limit of detection of *V. cholerae* O139, three *V. cholerae* O139-negative diarrheal stool samples were artificially inoculated with various concentrations (10^4 to 10^9 CFU/ml) of *V. cholerae* O139 grown in Trypticase soy broth (GIBCO) under stationary conditions at 37°C for 18 h. All three stool samples yielded positive results in the COAT only when the concentrations of *V. cholerae* O139 were 10^7 CFU and above. Therefore, it is likely that the seven culture-positive and COAT-negative samples had levels of *V. cholerae* O139 that were below the limit of detection of the COAT method. Use of an enrichment culture might increase the sensitivity of the COAT method (5).

Twenty fresh stool samples which tested positive and 10 stool samples which tested negative by COAT were frozen at −20°C. They were thawed out and retested 1 week later by COAT; identical results were obtained. This showed that freezing of samples did not have any adverse effect on detection of *V. cholerae* O139.

Compared with culture as the "gold standard," the COAT had a sensitivity of 92%, a specificity of 100%, a 100% positive predictive value, and a 95% negative predictive value. The test can be carried out in the field, hospital ward, or clinic, and the results are available within a few minutes. It is possible that the reagents can be put together in the form of a kit. Since this test cannot detect *V. cholerae* O1, it should be used in conjunction with the Cholera Screen test. However, efforts to devise a single test by combining MAbs to both *V. cholerae* O1 and O139 are under way.

This research was supported by the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The ICDDR,B is supported by aid agencies of the governments of Australia, Bangladesh, Belgium, Canada, Denmark, Japan, the Netherlands, Norway, Saudi Arabia, Sweden, Switzerland, the United Kingdom, and the United States; the Children's Fund, Development Program, and Population Fund of the United Nations; the World Health Organization; and the Ford and Sasakawa Foundations.

We thank Manzurul Haque for secretarial assistance.

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