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Rapid Screening of Fecal Samples for *Vibrio cholerae* by a Coagglutination Technique

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A coagglutination procedure for detecting *Vibrio cholerae* was applied directly to 125 watery fecal samples received in the laboratory for bacteriological culture; many of these were from suspected cases of cholera. Of 47 bacteriologically proved cases of cholera, 44 (93.6%) gave positive results by the coagglutination method. There was a good correlation between the coagglutination method, dark-field microscopy, and culture.

Prompt diagnosis of any infectious disease is always advantageous, and this is especially so for a condition like cholera, which has a rapid course of infection. Rapid methods of diagnosis like coagglutination have been found to be useful and successful in diseases like meningitis, streptococcal infections, and salmonella infections (7–9). With this in view, the coagglutination procedure was applied directly to watery fecal samples received in our laboratory. These samples were tested on receipt, or if immediate testing was not possible, they were kept at 2 to 4°C until the time of testing.

A 10% suspension of *Staphylococcus aureus* Cowan I cells was prepared (2). Antisera supplied by the National Institute of Cholera and Enteric Diseases, Calcutta, India, were used for sensitization. For sensitization, 0.1 ml each of the *V. cholerae* polyvalent and Ogawa serotype (the commonest serotype now encountered in India) antisera was added separately to 1 ml of the 10% staphylococcal suspension. The mixture was left at room temperature for 30 to 45 min and then centrifuged. The sensitized staphylococci in the deposit were resuspended in phosphate-buffered saline containing 0.1% sodium azide to give a final concentration of 2% sensitized cells. This suspension was found to be stable for many months at 4°C. A control to detect nonspecific agglutination was included with each specimen by using unsensitized Cowan I cells.

A standard strain of *V. cholerae* biotype El Tor, serotype Ogawa (AD12-og) obtained from the National Institute of Cholera and Enteric Diseases, Calcutta, India, was tested and found to give clear and strong clumping with Staphylococcus A cells sensitized with *V. cholerae* polyvalent and Ogawa antisera, but no reaction was seen with unsensitized cells.

Reagents were brought to room temperature before use. The test was performed in slides with ceramic rings or in cavity slides. One drop of the fecal sample was pipetted with a Pasteur pipette into each of three rings or cavities. One drop of each of the reagents, including *V. cholerae* polyvalent reagent, serotype Ogawa reagent, and unsensitized staphylococcal cells, respectively, were added and mixed well with a stick. Readings were taken in front of a viewing lamp after hand rotation for 2 min. The intensity of each reaction was graded 1+ to 4+ or negative, with a positive reaction giving a clear clumping.

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Of the 125 fecal samples, 41 gave a nonspecific positive reaction with unsensitized control reagent, and these were retested after absorption with staphylococcus A cells. Equal volumes of fecal sample and packed staphylococcal cells were mixed well and allowed to stand at room temperature for 15 min. This solution was centrifuged, and the supernatant was tested by the coagglutination test. Absorption in no way detracted from the positivity of a true positive reaction.

Dark-field microscopy studies were done on all fecal specimens from suspected cholera cases. If the result was positive, the dark-field immobilization test was carried out with *V. cholerae* polyvalent antiserum.

All specimens were inoculated onto blood agar, MacConkey agar, deoxycholate citrate agar, bismuth sulfite agar, and thiosulphate citrate bile salts sucrose agar. Two enrichment broths, Selenite-F Enrichment (BBL Microbiology Systems) and alkaline peptone water, were also inoculated. Selenite-F Enrichment was subcultured after 12 to 14 h onto MacConkey agar, and alkaline peptone water was subcultured after 4 h onto blood agar and MacConkey agar. The pathogens were identified by conventional biochemical tests and confirmed serologically by slide agglutination with specific antisera (3).

Of the 125 samples, 47 yielded *V. cholerae* serotype Ogawa. Of these 47, 44 gave positive coagglutination tests, and of the remaining 78 culture negative cases, 76 gave a negative coagglutination test as well. The sensitivity, specificity of positive predictive value, and specificity of negative predictive value of the coagglutination test were high (Table 1). These values were calculated by using culture results as the standard.

In 13 cases, we also performed the test on a 4-h alkaline peptone water culture of the stool; these cultures also gave strong and clear positive results and correlated well with the culture results. It was observed that when the test was performed on alkaline peptone water culture, only one nonspecific reaction was encountered, obviating the absorption step in the rest of the cultures.

The usual rapid diagnostic methods for cholera include light microscopic examination of a hanging drop or dark-field microscopic examination of a wet preparation of stool or both. Hanging-drop examination is, no doubt, very simple and rapid, but it is not of much value, especially if the material contains a mixed microbial flora (1). Dark-field microscopy, however, is more reliable in that it is easier to pick out the positive cases, and in combination with the immobilization technique with specific *V. cholerae* polyva-

TABLE 1. Comparison of results of coagglutination tests and culture of *V. cholerae*[a]

Test results for coagglutination test (no.)	Culture for *V. cholerae*	
	No. positive	No. negative
Positive (46)	44	2
Negative (79)	3	76

[a] Sensitivity, 44 of 47 (93.6%) for positive *V. cholerae* culture; specificity, 76 of 78 (97.4%) for negative *V. cholerae* culture; predictive value for positive coagglutination test results, 44 of 46 (95.7%); predictive value for negative coagglutination test results, 76 of 79 (96.2%).

lent antiserum (1), its specificity rates are high. The coagglutination test compares well with dark-field microscopy. Of the 125 samples tested, the coagglutination test, dark-field microscopy, and immobilization and culture were carried out in 51 cases. The coagglutination test was positive in all 29 culture-positive cases (100%), whereas dark-field microscopy and immobilization was positive in 27 cases (93.1%). It also proved to be highly specific for *V. cholerae* (Table 2). One case that was positive by coagglutination and dark-field microscopy and immobilization grew *Aeromonas* spp. in culture, and it is possible that *V. cholerae* was missed. Isolates of *Aeromonas*, including the above strain, were tested and found not to cross-react with the *V. cholerae* reagents in the coagglutination test.

TABLE 2. Results of coagglutination tests in comparison with culture showing specificity for *V. cholerae*

Pathogen	No. coagglutination positive[a]	No. coagglutination negative	No. culture positive
V. cholerae serotype Ogawa	44	3	47
Nonagglutinable vibrios	0	11	11
Aeromonas spp.	1	3	4
Shigellae spp.	0	5	5
Salmonella spp.	0	2	2
Salmonella typhimurium	0	2	2
EPEC	0	1	1
Plesiomonas shigelloides	0	2	2

[a] 0, None gave a positive reaction.

The direct coagglutination test described here is inexpensive, the reagents were easily prepared, and the reading of the test does not require any special training of laboratory personnel and thus can be performed in any small laboratory. In view of this and its high sensitivity and specificity, we recommend use of the coagglutination technique on fecal samples for rapid and specific diagnosis of cholera, which continues to be endemic in some parts of the world (3–6).

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