Evaluation of the Monoclonal Antibody-Based Kit Bengal SMART for Rapid Detection of *Vibrio cholerae* O139

**Synonym Bengal in Stool Samples**

FIRDAUSI QADRI,1,* JAFRUL A. K. HASAN,2 JABER HOSSAIN,1 ASHRAFUZZAMAN CHOWDHURY,1 YASMIN ARA BEGUM,1 TASNIM AZIM,1 LAWRENCE LOOMIS,2 R. BRADLEY SACK,† AND M. JOHN ALBERT1

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka 1000, Bangladesh,1 and New Horizons Diagnostics Corporation, Columbia, Maryland2

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A monoclonal antibody-based test, Bengal SMART, was developed for rapid detection of *Vibrio cholerae* O139 synonym Bengal directly from stool specimens. The test, which takes about 15 min to complete, was used to screen 189 diarrheal stool specimens. The results were compared with those of a monoclonal antibody-based coagglutination test (COAT) and the conventional culture methods used as the “gold standard” for detection of *V. cholerae* O139. The Bengal SMART test showed a sensitivity of 100% and a specificity of 97% in comparison with the gold standard. It also fared better than COAT, which had a sensitivity of 96% for rapid detection of *V. cholerae* O139 synonym Bengal. In the development of the Bengal SMART kit, MAb ICL12 (7) and a rabbit polyclonal serum against *V. cholerae* O139 were used (6). Here we report on the evaluation of Bengal SMART.

For evaluation of the effectiveness of the Bengal SMART kit in the detection of *V. cholerae* O139 in clinical specimens, single stool samples were obtained from 189 patients, most of whom had clinical cholera, who were treated at the Clinical Research and Service Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh, in Dhaka, Bangladesh, between September 1993 and April 1994. The stools collected were mainly watery (87%), but loose (6%) and mucoid (7%) stools were also tested. These were cultured for vibrios and other enteric pathogens as described previously (13). The Bengal SMART test was carried out blindly by a person different from the one doing the culture. After the stool samples had been cultured, the leftover samples were either utilized for the Bengal SMART test within 2 h (91 samples) or stored at −20°C for a maximum of 6 months and then tested (the remaining 98 samples).

Of the 91 samples screened by Bengal SMART immediately after collection, 31 tested positive for *V. cholerae* O139. Twenty-nine of these samples yielded *V. cholerae* O139 by culture. The two samples which were positive for *V. cholerae* O139 by SMART but did not yield the organism by culture were also negative for *V. cholerae* O1 by culture. (These two samples were not tested by Cholera SMART.) From the remaining samples, 17 *V. cholerae* O1 El Tor Ogawa strains; 2 non-O1, non-O139 *V. cholerae* strains; 7 *Campylobacter jejuni* strain; 7 enterotoxigenic *Escherichia coli* strains; and 1 *Shigella dysenteriae* type 1 strain were isolated. No recognized bacterial pathogens were identified from 34 stool samples.

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*Vibrio cholerae* O139 synonym Bengal, a newly recognized serogroup of *V. cholerae*, has recently caused epidemics of cholera in the Indian subcontinent, as well as in other Asian countries (1, 3, 4, 9). This organism is now considered to be the second most important etiologic agent of cholera (12), the first being *V. cholerae* O1. It is now believed that this serogroup of *V. cholerae* has probably initiated the eighth pandemic of cholera (11). We have produced monoclonal antibodies (MAbs) specific for the lipopolysaccharide antigen of *V. cholerae* O139 and have used them successfully for the detection of the pathogen in direct slide agglutination tests, motility inhibition tests, and indirect immunofluorescence tests (7). Two of these MAbs, ICL11 and ICL12, have also been used in a coagglutination test (COAT) after adsorption to *Staphylococcus aureus* Cowan 1 cells. COAT showed 92% sensitivity and 100% specificity for direct detection of *V. cholerae* O139 from fecal samples in comparison with the culture method used as the “gold standard” (8).

Recently, Hasan et al. (5) have developed and tested a highly sensitive and specific colloidal-gold-based colorimetric immunnoassay called Cholera SMART (for sensitive membrane antigen rapid test) (New Horizons Diagnostics Corporation, Columbia, Md.) for the direct detection of *V. cholerae* O1 in clinical specimens, and this test takes only 15 min to complete. The test uses a MAb specific to the A factor of *V. cholerae* O1 lipopolysaccharide (2) and a high-titer polyclonal anti-*V. cholerae* O1 antibody. The principle of the test is as follows. A specimen suspected of containing *V. cholerae* O1 is reacted with the colloidal-gold-labeled MAb. If *V. cholerae* O1 is present in the specimen, it complexes to the *V. cholerae* O1 MAb. The complex diffuses and is captured and concentrated by the polyclonal antibody-coated solid-phase matrix. It then appears to the naked eye as a pink-to-red test spot developing from the deposition of colloidal gold. In the absence of *V. cholerae* O1, the complex does not form and hence no pink-to-red color appears in the test spot. The Bengal SMART kit, modeled after Cholera SMART (New Horizons Diagnostics Corporation), was developed for the detection of *V. cholerae* O139 synonym Bengal. In the development of the Bengal SMART kit, MAB ICL12 (7) and a rabbit polyclonal serum against *V. cholerae* O139 were used (6). Here we report on the evaluation of Bengal SMART.

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Of the 98 samples that had been stored at −20°C before
testing, 56 were positive for *V. cholerae* O139 by the Bengal SMART kit. By culture, however, only 55 of these samples yielded *V. cholerae* O139. The one sample positive for *V. cholerae* O139 by Bengal SMART but not by culture was also negative for *V. cholerae* O1 by culture and the Cholera SMART test (5). Of the rest, 10 samples yielded *V. cholerae* O1 by culture and were positive for the presence of cholera toxin genes by PCR. They were negative for *V. cholerae* O1 and other enteric bacterial pathogens by culture. Of these, one sample was also found to be negative when tested by Cholera SMART.

Three specimens were positive for the presence of cholera toxin genes by PCR. They were negative for *V. cholerae* O1 and other enteric bacterial pathogens by culture. Of these, one sample was also found to be negative when tested by Cholera SMART.

Other enteric bacterial pathogens cultured included 27 *V. cholerae* O1 strains; 2 *V. cholerae* non-O1, non-O139 strains; 10 enterootoxigenic *E. coli* strains; 1 C. jejuni strain; and 1 J. dysenteriae type 1 strain. Sixty-one samples were negative for known enteric pathogens.

TABLE 1. Detection of *V. cholerae* O139 synonym Bengal in stool samples by Bengal SMART test versus conventional cultures

<table>
<thead>
<tr>
<th>Bengal SMART result</th>
<th>No. of samples with indicated culture result for <em>V. cholerae</em> O139 synonym Bengal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>84</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Positive negative</td>
<td>3</td>
</tr>
<tr>
<td>Negative positive</td>
<td>102</td>
</tr>
</tbody>
</table>

<table>
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<tr>
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<th>Positive</th>
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<td>0</td>
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</tr>
</tbody>
</table>

A total of 189 stool samples were tested.

Sensitivity was 100% (84 of 84) for positive *V. cholerae* O139 culture results, specificity was 97% (102 of 105) for negative *V. cholerae* O139 culture results, and overall agreement was 98% (106 of 189). Bengal SMART had a positive predictive value of 97% (84 of 87) and a negative predictive value of 100% (102 of 102).

Three specimens were positive for the presence of cholera toxin genes by PCR. They were negative for *V. cholerae* O1 and other enteric bacterial pathogens by culture. Of these, one sample was also found to be negative when tested by Cholera SMART.

Other enteric bacterial pathogens cultured included 27 *V. cholerae* O1 strains; 2 *V. cholerae* non-O1, non-O139 strains; 10 enterootoxigenic *E. coli* strains; 1 C. jejuni strain; and 1 J. dysenteriae type 1 strain. Sixty-one samples were negative for known enteric pathogens.

Bengal SMART test results for fresh and frozen samples were pooled and compared with culture results (Table 1). Compared with culture as the gold standard, Bengal SMART showed a sensitivity of 100%, a specificity of 97%, a positive predictive value of 97%, and a negative predictive value of 100%. However, since the three Bengal SMART-positive but culture-negative stool samples were tested positive for cholera toxin by PCR, these samples are not really false positives, suggesting that the specificity of the test is higher. The MAb, ICL12, used in the development of Bengal SMART had previously been tested for specificity by being screened against a variety of organisms, including *V. cholerae* O1, and had been found to be specific (7, 8). The stool specimens that were Bengal SMART negative showed a variety of normal enteric bacterial flora (*E. coli* and Proteus, Klebsiella, Enterobacter, and *Pseudomonas* spp., etc.) and bacterial pathogens (Table 1) on culture. Since the presence of these organisms did not give rise to positive test results, it is safe to assume that Bengal SMART is specific for *V. cholerae* O139.

Seventy-five stool specimens (47 fresh and 28 frozen) tested by Bengal SMART were also tested by COAT (preparred with MAb ICL12). Among these samples, 55 were positive by Bengal SMART, and of those 55 samples, 51 were positive by COAT and 53 were culture positive for *V. cholerae* O139. Thus, COAT showed a sensitivity of 96%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 92% compared with culture. Two samples that were culture negative but Bengal SMART positive were also negative by COAT. These were the same samples which tested positive for ctxA and were negative for *V. cholerae* O1 by culture. These results are not surprising because Bengal SMART is about 20 times more sensitive than COAT for detection of *V. cholerae* O139 (detection limit, 5 × 10⁵ CFU/ml for Bengal SMART versus 1 × 10⁷ CFU/ml for COAT) (8) (New Horizon Diagnostics).

These results showed that Bengal SMART is highly sensitive and specific for detection of *V. cholerae* O139 in fecal samples. It will find application in identifying the O139 serogroup in outbreaks and epidemics, and in tracking the spread in areas of endemicity. It is more sensitive than either culture or COAT. The three specimens that were positive by Bengal SMART but negative for *V. cholerae* O139 by conventional culture techniques probably contained *V. cholerae* O139, because these samples were negative for *V. cholerae* O1 by culture and testing of one sample by Cholera SMART also gave a negative result. The fact that these specimens were also positive for ctxA by PCR gives additional support to the possibility that they indeed contained *V. cholerae* O139. Since the SMART technique detects both viable and nonviable bacteria, it is more sensitive than culture methods, which detect only viable organisms. Bengal SMART is a rapid test, yielding results within 15 min of testing, and both fresh and frozen stool samples can be used. Moreover, the Bengal SMART kit does not require refrigeration and is, therefore, suitable for use in field settings. It is thus a useful addition to Cholera SMART for detection of both etiological agents of cholera. A SMART kit which will detect both *V. cholerae* O139 and *V. cholerae* O1 by incorporating MAb to both pathogens is under evaluation. It should be borne in mind that Cholera SMART and Bengal SMART are designed for detection of etiological agents of cholera only. Other serogroups of *V. cholerae* which may cause diarrhea will have to be detected by conventional culture techniques.

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