One-Step Immunochromatographic Dipstick Tests for Rapid Detection of *Vibrio cholerae* O1 and O139 in Stool Samples

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We describe the development and evaluation of a rapid diagnostic test for *Vibrio cholerae* O1 and O139 based on lipopolysaccharide detection using gold particles. The specificity ranged between 84 and 100%. The sensitivity of the dipsticks ranged from 94.2 to 100% when evaluated with stool samples obtained in Madagascar and Bangladesh. The dipstick can provide a simple tool for epidemiological surveys.

Vibrio cholerae strains belonging to the O1 and O139 serogroups are capable of causing epidemic and pandemic cholera. The O1 serogroup is subdivided into two serotypes, Ogawa and Inaba. Serogroup O139, which appeared in India in 1992, has spread rapidly throughout Asian countries and is considered to be the potential eighth pandemic strain of cholera. Prompt diagnosis of cholera is of key importance to initiate effective therapy and to institute proper epidemiological measures. There are definitive indications that the incidence of this serogroup is on the rise in India and Bangladesh (17).

Several rapid diagnostic tests for cholera have been described. Some detect the cholera toxin (2, 19). The others detect the lipopolysaccharide (LPS) antigen of *V. cholerae* O1 (3, 6, 8, 12, 16) or O139 (1, 10, 14). Recently, a multistep colloidal-gold-based colorimetric immunoassay known as SMART was also developed for direct detection of *V. cholerae* O1 (9, 11) or *V. cholerae* O139 (15) in stool specimens and has demonstrated 95% sensitivity and 100% specificity for O1 strains (11) and 100% sensitivity and 97% specificity for O139 strains (15).

In our effort to develop a conjugate vaccine that targets *V. cholerae* O1 and O139, we have developed monoclonal antibodies specific to *V. cholerae* O1 or O139 LPS (4, 5). Here we have exploited the specificity of the monoclonal antibodies to develop rapid diagnostic tests for cholera O1 or O139 using colloidal gold particles and based on a recently optimized (7) one-step, vertical-flow immunochromatography principle (13). The detection threshold with purified LPS was 10 ng/ml for *V. cholerae* O1 and 50 ng/ml for *V. cholerae* O139. The dipsticks were stable after storage for 21 days at 60, 4, −20, and −80°C.

We have evaluated the sensitivity and specificity of the rapid dipstick tests in the laboratory setting and in two areas of cholera endemicity, namely, in Madagascar and in Bangladesh. The specificity was assessed using 14 pure cultures of *V. cholerae* non-O1/non-O139 and 16 strains belonging to six other species of the genus *Vibrio* (*V. alginolyticus*, *V. fluvialis*, *V. parahaemolyticus*, *V. furnissii*, *V. hollisae*, and *V. mimicus*), seven

strains of *Aeromonas* species (*A. caviae*, *A. enteropelogenes*, *A. hydrophila*, *A. sobria*, and *A. trota*), two strains of *Plesiomonas shigelloides*, and two strains of *Campylobacter jejuni*. Additionally, eight strains of *Yersinia pseudotuberculosis*, 10 strains of *Yersinia enterocolitica*, 35 other strains of *Yersinia* belonging to seven species, and another 47 strains belonging to 11 other genera of *Enterobacteriaceae* were included. The specificity of both dipsticks was 100% for all bacterial cultures. When tested for sensitivity, O1 dipsticks were positive with all 12 strains of *V. cholerae* O1 (100%) and O139 dipsticks were positive with 17 of the 19 strains of *V. cholerae* O139 (89.5%). A minimum of ca. 10^7 CFU of *V. cholerae* O1/ml or 10^6 CFU of *V. cholerae* O139/ml is required to give an unequivocal positive reaction.

In Madagascar, the cholera O1 dipsticks were tested on 140 frozen stools samples or on rapid cultures (6 h, 37°C) of stool collected in the field on filter paper. Sixty-five samples were dipstick and culture positive, 1 sample was dipstick negative and culture positive, 3 samples were dipstick positive and culture negative, and 71 samples were negative by both tests (Table 1). From the three dipstick-positive and culture-negative samples, one was positive for *Shigella* spp. The specificity in this field trial was 96%, and the sensitivity was 98.5%. The specificity of O139 dipsticks was also tested with the same 74 noncholera samples. Seventy-one out of the 74 samples tested negative (96%). The three samples which were positive by the dipstick test and negative in conventional culture were positive for *Shigella*.

In Bangladesh, fresh stool samples from suspected cholera patients were cultured for vibrios and other enteric pathogens as described previously (14, 15). Frozen stool samples in which the etiology was known were made available for this study from the specimen bank of the International Centre for Diarrhoeal Diseases Research, Bangladesh. The dipstick test was performed simultaneously by introducing either the O1 or the O139 dipsticks into 200 µl of stool.

For the O1 dipstick evaluation, 102 stool samples were used. Forty-nine were dipstick and culture positive, 8 were dipstick positive and culture negative, 3 were dipstick negative and culture positive, and 42 were negative by both tests (Table 2). The sensitivity of the O1 dipstick compared to culture was 94.2% with a specificity of 84%. We further analyzed the eight

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TABLE 1. Detection of *V. cholerae* O1 in 140 stool samples or precultures, by O1 dipstick test versus conventional culture (Madagascar)

Bacteriological culture	No. of specimens with O1 dipstick test result[a]		Total no. of specimens
	Positive	Negative	
Positive	65[b]	1	66
Negative	3[c]	71	74
Total	68	72	140[d]

[a] Sensitivity was 98.5%; specificity was 96%.
[b] The presence of *V. cholerae* serogroup O1 was confirmed in 24 stool specimens and 41 preculture specimens. One preculture was negative.
[c] One stool specimen was *Shigella* positive.
[d] Of the 140 samples, 98 were stool samples and 42 were precultures.

stool samples which were O1 dipstick positive but O1 culture negative; six were negative for *V. cholerae*, one was positive for *V. cholerae* O139 and *Proteus* spp., and another was positive for *Pseudomonas* spp. All three stool samples which were negative by the dipstick test were positive for *V. cholerae* O1. One of the reasons for such a result might be that the amount of LPS in these stool samples is lower than threshold levels but such stool samples were not many in numbers.

For the O139 dipstick evaluation, of the 158 stool samples, 91 were dipstick and culture positive, 5 were dipstick positive and culture negative, and 60 were negative by both dipstick and culture (Table 3). The sensitivity of the O139 dipstick compared to culture was 100%, with a specificity of 92.5%. We further analyzed the five stool samples which were O139 dipstick positive but O139 culture negative and found three samples were positive for *V. cholerae* O1 Inaba by culture while the other two were negative for vibrios.

In the evaluation in Madagascar as well as that in Bangladesh there were few samples which were positive by the dipstick test but negative by culture for both the O1 and O139 strains. We did not have a method to prove whether these results were true or false positives as we did not perform a PCR on these samples. However, it is possible that some of these stool samples lacked live organisms but contained enough LPS to react with the dipsticks, due to prior treatment with antibiotics or long delay and bad field conditions during the conveying of the samples in Madagascar. Plans are under way to conduct a more rigid evaluation to understand whether these were false-positive results.

TABLE 2. Detection of *V. cholerae* O1 in fresh and frozen stool samples by O1 dipstick test versus conventional culture (Bangladesh)

Bacteriological culture	No. of specimens with O1 dipstick test result[a]		Total no. of specimens
	Positive	Negative	
Positive	49	3	52
Negative	8	42	50
Total	57	45	102[b]

[a] Sensitivity was 94.2%; specificity was 84%.
[b] Of the 102 samples, 77 were fresh and the remaining 25 were frozen for various periods.

TABLE 3. Detection of *V. cholerae* O139 in fresh and frozen stool samples by O139 dipstick test versus conventional culture (Bangladesh)

Bacteriological culture	No. of specimens with O139 dipstick test result[a]		Total no. of specimens tested
	Positive	Negative	
Positive	91	0	91
Negative	5	62	67
Total	96	62	158[b]

[a] Sensitivity was 100%; specificity was 92.5%.
[b] Of the 158 samples, 100 were fresh and the remaining 58 were frozen for various periods.

In summary the importance of an efficient cholera surveillance system continues to be stressed by World Health Organization with regard to improving risk assessment of potential cholera outbreaks (18). Further, in outbreak situations, a quick diagnosis of cholera is essential for mobilization of resources for treatment and containment of the outbreak. Therefore, the need for sensitive and specific diagnostic tests that can be utilized by minimally skilled personnel and that require negligible laboratory infrastructure is very real. We embarked on this study to fulfill this need, with our priority being the development of a bedside detection test that can be performed by any health care worker and that comes in a format ideally suited for a resource-poor setting.

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