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Field evaluation of Crystal VC® Rapid Dipstick test for cholera during a cholera outbreak in Guinea-Bissau

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Summary

OBJECTIVES To evaluate performance characteristics and ease of use of the new commercially available Crystal VC® Rapid Dipstick (VC) test (Span Diagnostics, India) for *Vibrio cholerae* O1 and O139.
METHODS Whole stool was collected from patients presenting to a hospital cholera ward during a 2008 epidemic in Guinea-Bissau. The VC test on stool samples was conducted on-site; samples were subsequently stored in Cary-Blair transport media and sent to the Centers for Disease Control and Prevention for diagnostic testing by culture and polymerase chain reaction (PCR). In addition, four local laboratory technicians who were unfamiliar with the test were provided with stool samples, the VC test kit, and simple written instructions and asked to perform the test and interpret results.
RESULTS A total of 101 stool specimens were collected and tested. Compared with PCR, the test was 97% sensitive and 71–76% specific. Laboratory technicians in Bissau performed the test and interpreted results correctly using only simple written instructions.
CONCLUSIONS The VC test may be useful for cholera diagnosis in outbreak situations where laboratory capacity is limited.

keywords cholera, vibrio, rapid test, dipstick, Guinea-Bissau, diarrhoea

Introduction

Epidemic cholera occurs in areas with poor water and sanitation, where access to laboratory and medical services is often limited. In 2007, 53 countries reported 177 963 cholera cases and 4031 cholera deaths to the World Health Organization (WHO). Countries in sub-Saharan Africa reported more than 93% of the cases and 99% of the deaths (WHO 2008). Because of the epidemic potential of cholera and the high case-fatality rate among patients who do not receive proper treatment, rapid identification of initial cases in an epidemic is critical for mobilization of a timely public health response and disease containment measures.

Cholera diagnosis is usually confirmed with a positive bacterial culture, a process that takes 2–3 days and requires trained technicians, specialized reagents, and a functioning laboratory. Diagnostic confirmation may be delayed when laboratory capacity is limited or when affected areas are remote. In 2003, the Institut Pasteur developed a rapid dipstick test for the detection of *Vibrio cholerae* from stool specimens. The test is based on immunochromatography and colorimetric reporting, and detects *V. cholerae* O1 and O139 antigens binding to antibodies fixed on a nitrocellulose strip. In several studies, the test was shown to be 94–100% sensitive and 84–100% specific (Bhuiyan *et al.* 2003; Nato *et al.* 2003; Wang *et al.* 2006); in another study, the test was 93–94% sensitive and 67–76% specific (Kalluri *et al.* 2006). Through a licensure agreement with Span Diagnostics (India), the test was recently made commercially available at low cost as the Crystal VC® Rapid Dipstick (VC) test.

In Guinea-Bissau, a small West African country of approximately 1.7 million people, more than half of the population lacks access to safe drinking water, and sanitation is poor (WHO & UNICEF Joint Monitoring Programme 2008; UNICEF At a glance 2008; UNICEF Country profile 2008). Devastating cholera epidemics have

occurred in Guinea-Bissau in 1994, 1996, 1997, 2002, and 2005, and smaller outbreaks have taken place during most of the intervening years (Gunnlaugsson *et al.* 2000; Rodrigues *et al.* 2000; Einarsdottir *et al.* 2001; WHO 2003, 2006, 2008).

In May 2008, a new cholera epidemic began in Guinea-Bissau and lasted until mid-November, resulting in at least 13 921 reported illnesses and 221 deaths (UNOCHA 2008). Confirmation of the growing epidemic was delayed for many weeks due to lack of electrical power at the national laboratory. In late July, *V. cholerae* O1 (biotype El Tor, serotype Ogawa) was isolated from stool samples after they were sent to a reference laboratory in a neighbouring country. During August and September 2008, while the epidemic was ongoing, a team of epidemiologists from the US Centers for Disease Control and Prevention (CDC) and the Ministry of Health of Brazil, assisted by hospital workers from the municipal hospital in Bissau, evaluated the sensitivity, specificity, positive and negative predictive values of the VC rapid diagnostic test, and assessed its ease of use by local technicians naïve to the testing procedure.

Methods

Patient enrolment

Patients presenting to the cholera ward at Simão Mendes National Hospital (HNSM) were provided with a plastic cup for stool collection. Patients were asked to defecate directly into the cup. Information was collected on patient age, sex, date of symptom onset, stool quality, and treatment with antibiotics before hospital admission.

Rapid test procedure

The VC test was carried out as described per the manufacturer's instructions, included in the test kit. (All procedures included in the evaluation of sensitivity, specificity, positive and negative predictive value of the rapid test were carried out by one of two authors on this paper, each with graduate-level laboratory technical training.) Briefly, approximately 200 μl of faeces were transferred from the sample cup to a clean test tube using the plastic dropper included with the test kit. Semisolid samples were diluted with saline if necessary. A VC test dipstick was placed in the test tube such that approximately the last centimetre of the strip was immersed in the faeces. After 10 min (or upon appearance of the positive control band), the dipstick was removed and test results were read. Tests were judged as positive, negative, or indeterminate (ambiguous results) for *V. cholerae* O1 and for *V. cholerae* O139.

Sample collection, transport, and laboratory testing

Two sterile polyester swabs were soaked briefly in each stool sample within 6 h of sample collection, and were stored in Cary-Blair transport media. Samples were sent to the Enteric Diseases Laboratory at CDC within 1 week of collection. On arrival at CDC, specimens were plated onto thiosulphate citrate bile salt sucrose (TCBS) medium or enriched in alkaline peptone water broth for 6–8 h at 37 °C, and subsequently plated to TCBS and incubated overnight at 37 °C. Yellow (sucrose +) colonies suggestive of *V. cholerae* were inoculated on blood agar for serologic testing by slide agglutination in serogroup 0139 and serogroup O1 serotype-specific antisera (CDC 1999). All clinical specimens were screened by polymerase chain reaction (PCR) for the presence of cholera toxin (*ctxA*) and biotype-specific (*tcpA*) genes, and species-specific gene sequences (Fields *et al.* 1992; Keasler & Hall 1993; Ghosh *et al.* 1997; Nandi *et al.* 2000).

Data analysis

Results from the VC test were recorded on patient hospital forms and entered into a Microsoft Access database. Sensitivity (SN), specificity (SP), positive (PPV) and negative (NPV) predictive values of the VC test were calculated. Due to unforeseen problems with culture viability, PCR, rather than culture, was used as the gold standard for comparison. Because some VC tests yielded indeterminate results, SP, NPV and PPV are reported as a range of values, calculated by allowing the indeterminate VC test results to be either concordant or discordant with PCR results.

Ease of use of the test

Simple, one-page illustrated instructions for carrying out the VC test were written in Portuguese. The test kit, instructions, and five stool samples collected from patients presenting to HNSM were brought to three local clinics, all of which had at least one laboratory technician. The technicians were asked to read the instructions, perform the VC test on one or more specimens, and evaluate the samples as positive or negative for *V. cholerae* O1 or O139. Study authors observed the procedure; however, no assistance or additional explanations were provided to the technicians.

Results

Patient characteristics

Stool samples were collected from 101 patients presenting to the HNSM cholera ward between 29 August and 10

September 2008. All patients experiencing symptoms of cholera, including self-reported diarrhoea and/or vomiting and who were able to produce stools were considered eligible for the study. Of 100 patients whose sex was reported, 48 (48%) were female. Median patient age was 27 years; range 2–78 years. 18 (21%) patients reported taking antibiotics for their illness at least once before presentation at HNSM. 30 (33%) of patients reported that someone at home was experiencing a similar illness. 73 (89%) of 82 patients who reported stool type had 'rice water' stools, while 1 (1%) reported bloody stools and 8 (10%) reported mucoid stools.

Rapid dipstick test characteristics

Cary-Blair samples were sent from Guinea-Bissau to CDC in two separate shipments on 3 September and 13 September 2008. The first shipment contained 44 samples, and the second contained 57 samples. Overall, 26 (26%) of the 101 samples were VC test-negative; 73 (72%) were VC test-positive; and two (2%) were VC test-indeterminate (Table 1).

For reasons that are unclear, although the 57 samples in the second shipment sent to CDC were collected using the same methods as the initial shipment of 44 samples, *V. cholerae* O1 was isolated by culture from 29 (66%) of the samples in the first shipment and from only seven (12%) samples in the second shipment. Shipment conditions may have compromised sample quality; among the 50 culture-negative samples in the second batch, 31 (62%) were PCR-positive, and 30 (97%) of these were VC test-positive.

Because of the unknown problem with sample viability, PCR, rather than culture, was treated as the diagnostic gold standard for all samples. Among 101 samples with VC and PCR results, VC test characteristics when compared with PCR results were: SN 97%; SP 71–76%; PPV 87–89%; NPV 92–93% (Table 1). Insufficient numbers of tests were performed to examine predictors of false positive or false negative VC results, as compared with PCR results.

Table 1 Comparison of rapid dipstick (VC) and polymerase chain reaction (PCR) results for 101 stool samples sent for testing, cholera outbreak, Guinea-Bissau, 2008

	Dipstick			
PCR	VC-positive	VC-negative	VC-indeterminate	Total
PCR-positive	65	2	0	67
PCR-negative	8	24	2	34
Total	73	26	2	101

None of the samples were positive for *V. cholerae* O139 by the VC test, by culture, or by PCR.

Ease of use of the rapid dipstick test

Four local laboratory technicians performed 10 rapid tests on five stool samples. After reading the directions, the time taken by the laboratory technicians to complete the first test was approximately 10 min. For the two technicians who performed more than one test, the time taken for each successive test was reduced to approximately 5 min. All laboratory workers reported that the test was easy to perform and the directions were simple to follow.

The primary study investigators assessed four of the five samples provided to the local laboratory technicians as VC test-positive for *V. cholerae* O1, and one as indeterminate (Table 2). Two of the four local laboratory technicians performed the VC test on the indeterminate stool sample; one interpreted the test as negative and the other interpreted the test as positive for *V. cholerae* O1. The other four samples were assessed as VC test-positive for *V. cholerae* O1 by all four local laboratory technicians who tested them (a total of 10 tests). All five samples were stored in Cary-Blair media and evaluated for *V. cholerae* by PCR at CDC; two samples (including the sample that was VC test-indeterminate) were negative; three were positive for *Vibrio cholerae* O1 (Table 2).

Discussion

During a cholera epidemic in Guinea Bissau, we evaluated the performance and ease of use of a newly commercialized rapid (VC) test for the detection of *Vibrio cholerae* O1 and O139. Because cholera frequently occurs in remote regions where collection and transport of stool specimens to a diagnostic laboratory is challenging, a simple, low-cost, robust and reliable diagnostic test can help

Table 2 Authors' assessment and the assessment of four laboratory technicians of rapid dipstick (VC) test results following performance of the test by technicians

Sample ID	Authors' assessment	T1	T2	T3	T4	PCR results
1	Indeterminate			(+)	(−)	(−)
2	(+)			(+)	(+)	(−)
3	(+)	(+)			(+)	(+)
4	(+)			(+)	(+)	(+)
5	(+)		(+)		(+)	(+)

Polymerase chain reaction (PCR) results are shown on the right. Cholera outbreak, Guinea-Bissau, 2008. (+); positive result, *V. cholerae* O1. (−); negative result.

inform early decision-making by public health authorities. We found that the VC test was highly sensitive and moderately specific for detection of *V. cholerae* O1 in an epidemic setting, and that local laboratory technicians were able to correctly apply the test with ease, using only simple written instructions.

Oral and intravenous rehydration is the recommended treatment for acute watery diarrhoea with dehydration regardless of aetiology; thus a rapid diagnostic test adds little to the clinical management of patients suffering from severe cholera. However, from a public health perspective, the detection of cholera cases in a new geographic region should trigger rapid preventive actions that minimize disease spread and resultant morbidity and mortality. These actions include community mobilization to encourage patients with acute watery diarrhoea to seek immediate medical care, engagement of health care providers to ensure that appropriate surveillance, reporting, and treatment guidelines are followed, promotion of protective behaviours related to food and water handling, hygiene, and sanitation, and ensuring that a sufficient supply of materials for cholera treatment, such as oral rehydration salts and antibiotics, are available. Delayed recognition of cholera outbreaks can lead to longer periods of time during which the disease spreads unchecked, resulting in more cases and higher case-fatality rates.

Because of the severity of cholera and the potential for rapid disease spread, the ability of a rapid diagnostic test to correctly identify true cases (sensitivity) is important. However, false positive test results could cause unnecessary mobilization of resources for disease control in areas that are already resource-poor, making the specificity of such a test similarly desirable. The VC test displayed high sensitivity; however, a specificity of approximately 71–76% means that one in every four patients without cholera would be misclassified as cholera-positive. Because of this, the VC test should not be considered as definitive confirmation of *V. cholerae* infection, particularly on a patient-by-patient basis. Rather, the test's utility will be greatest when an outbreak of cholera is suspected, and several patients are tested simultaneously. A predominance of positive test results in this situation would strongly support *V. cholerae* as the causative etiologic agent of the outbreak. However, even when this occurs, attempts should always be made to ensure that at least some cases are culture-confirmed, to validate the results and to permit antimicrobial susceptibility testing that can inform local treatment guidelines.

This study was conducted during a cholera outbreak in Guinea-Bissau, at a time when most of the patients admitted to the cholera ward with acute watery diarrhoea did, in fact, have cholera. Because the PPV of a test is dependent on the population prevalence of disease, the PPV will be reduced and will result in a higher proportion of false positive results if the test is used for periodic surveillance or as a screen for patients presenting with diarrhoea in a low cholera-prevalence region. Thus, while the test may be used to detect disease at the start of an outbreak, it should not be used to confirm the end of an outbreak. The VC test may be most useful in regions where no alternative diagnostic test exists, such as in refugee camps or remote regions where laboratory capacity is inadequate. In all cases, attempts should be made to confirm cases with standard laboratory methods.

The VC test kit cost approximately $2 per test as of late 2008; however, shipping and duty costs (from India to the US) add an additional $1–2 per test, making the total cost per test close to $4. This places the test expense below the estimated cost of culture confirmation and serotyping for *V. cholerae*, which is approximately $6–8 per test (M. Parsons and C. Bopp, personal communication). In addition, the test kits can be stored at ambient temperature for long periods of time, an advantage in areas where electricity is limited.

Our evaluation of this test had several limitations. Although the test has the capacity to detect *V. cholera* O1 and O139, we were not able to evaluate its sensitivity for *V. cholerae* O139 as this serogroup was not present among the patient population. We were also unable to enroll enough patients who had been treated with antibiotics to fully assess the relative sensitivity of the VC test, as compared to PCR, among these patients.

Illness duration among the patients enrolled in our study ranged from 0 to 2 days before administration of the rapid test. We do not know whether the test will be as sensitive among patients with illness onset >2 days before testing. While there were few indeterminate VC test results in this study, it is important to realize that indeterminate results will occur and will not provide useful information. For all samples in this study with ambiguous VC test results, PCR results were negative. This suggests that ambiguous VC test results may be more likely to be true negatives than true positives; however, the sample size is small. In addition, the small sample of local laboratory technicians who performed the VC test with only written instructions did not permit us to fully assess the ease of use of the test in 'real life' conditions. Users naïve to the test might experience difficulties that were not observed in this study.

In summary, the Crystal VC® Rapid Dipstick test showed high specificity and moderate sensitivity for detection of *V. cholerae* O1 in an epidemic setting, and good ease of use in this study. It may be useful for early confirmation of cholera outbreaks where laboratory capacity is limited.

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