Performance and utility of a rapid diagnostic test for cholera: notes from Haiti

Jacques Boncy a, Emmanuel Rossignol a, Georges Dahourou b, Marisa Hast c, Josiane Buteau a, Magalie Stanislas a, Daphne Moffett c, Cheryl Bopp d, S. Arunmozhi Balajeec,*

a National Public Health Laboratory, Ministry of Public Health and Population, Port-au-Prince, Haiti
b Centers for Disease Control and Prevention, Port-au-Prince, Haiti
c Division of Global Disease Detection and Emergency Response, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, USA
d Division of Foodborne, Water and Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

Abstract
The present study details work done at the National Public Health Laboratory in Haiti (LNSP), comparing the results of a cholera rapid diagnostic test (RDT) with culture-based methods. As of October 21, 2011, 644 specimens were tested by both RDT and culture-based method at the LNSP. The sensitivity and specificity of RDT were 95% and 80%, respectively, with a positive predictive value of 89% and negative predictive value of 91%. In resource-limited settings, the RDT has good utility and should be considered as part of the laboratory testing algorithm.

Keywords:
Cholera
RDT
Crystal VC

Rapid diagnostic tests (RDTs) and near point-of-care tests are revolutionizing the field of infectious disease diagnostics by offering simple, quick, and often economical platforms for detection of diseases of public health importance, including cholera, malaria, dengue, and meningitis (Bissonnette and Bergeron, 2010; Urdea et al., 2006). Recently, advocacy for the employment of cholera RDTs in limited-resource field conditions on stool samples to reduce the spread of the disease has been made (Dick et al., 2012). Numerous studies have demonstrated the utility of 1 cholera RDT in outbreaks, the Crystal Vc (Crystal VC, Code No. 25995A, Span Diagnostics Ltd, Surat, India), both in laboratory and field settings. This study adds to the body of knowledge and is unique in that it was conducted in Haiti where cholera has been declared for the first time in decades.

Cholera was first suspected in Haiti in 2 of 10 administrative departments (Artibonite and Centre) in October 2010 and subsequently laboratory-confirmed at Haiti’s National Public Health Laboratory (LNSP) in the nation’s capital Port au Prince on October 21, 2010 (CDC, 2010). Immediately following the laboratory confirmation in Artibonite and Centre departments, the Crystal VC tests (RDTs) were made available at every departmental laboratory by LNSP for continued case detection (Kwalling et al., 2011). Crystal VC RDT detects Vibrio cholerae O1 and 139 in stool samples and has been shown to have sensitivities and specificities in the range of 92–97% and 71–89%, respectively (Alam et al., 2010; Harris et al., 2009; Mukherjee et al., 2010; Wang et al., 2006).

Early in the cholera outbreak, all departmental laboratories were encouraged to perform the Crystal VC test as a screening test and send a proportion of specimens to LNSP for further culture-based testing (Fig. 1). A visual job-aid was created in French for the correct utilization of RDTs and was disseminated to all the departmental laboratories. All departmental laboratories were requested to routinely send a proportion of aseptically collected rice water stool specimens to LNSP. At the departmental level, up to 10 stool specimens from suspect, individual cholera cases were tested by the RDTs, and if more than 3 were positive, stool specimens were sent to LNSP for testing. Specimens arrived at the LNSP either as freshly collected stool samples or in Cary Blair (CB) medium. At the LNSP, specimens were worked up for RDT, culture, and antimicrobial susceptibility testing. Although Crystal VCs were available both at the departmental and the reference laboratory, the present study details data generated at LNSP.

Stool specimens were collected and transported to LNSP within 24 to 48 hours accompanied by a specimen referral form. At the LNSP, specimens were usually tested by RDT and cultured the day of arrival in the lab and were stored at ambient temperature until discarded. As of October 21, 2011, 1439 specimens were received both as stool (n = 644) and in CB transport media (n = 795); specimens received in CB medium were not tested by RDT as these required an additional enrichment step.

Specimens were inoculated onto Thiosulfate-Citrate-Bile-Sucrose Agar medium, incubated overnight at 37 °C, and suspect isolates...
were identified using standard methods (Talkington et al., 2011). Serogroup and serotype were determined by agglutination using specific antisera (Lee Laboratories, Franklin Lakes, NJ, USA). Six hundred and forty-four of these stool specimens were available for testing by RDT. The dipstick test employs monoclonal antibodies specific to O1 and 139 based on a 1-step, vertical flow immunochromatographic method. To perform the RDT assay, 200 μL of fresh stool specimens were transferred to a tube, and the dipstick was placed in the specimen and incubated at room temperature. The tests were defined as positive when both the test line and control line appeared on the test strip, with results read within 15 minutes per manufacturer’s instructions.

Of the 644 stool specimens tested by culture and RDT, 381 specimens were positive by both culture and RDT-based methods, and 196 were negative by both tests (Table 1). Nineteen specimens that were positive by culture were negative by RDT. Forty-eight specimens that were negative by culture were positive by RDT. Results demonstrated that the sensitivity and specificity of RDT in comparison with culture were 95% and 80%, respectively, with a positive predictive value of 89% and a negative predictive value of 91% with a 95% confidence interval.

The manufacturer’s website publishes a 94% and 84% sensitivity and specificity, although it is not clear how many and what type of samples were tested and under what conditions the testing was performed (Span Diagnostics, 2010). A recent study in India tested 212 specimens using the Crystal VC RDT and reported a 92% and 73% sensitivity and specificity, respectively, as compared to conventional methods (Mukherjee et al., 2010). Another study in Guinea-Bissau tested 101 specimens and showed that the Crystal VC RDT was 97% sensitive and 71–76% specific when compared to a polymerase chain reaction (PCR)–based detection method (Harris et al., 2009).

The RDT assay in the present study missed identifying 19 of the cases that were positive by culture, thus decreasing its sensitivity. This discrepancy could be due to the higher limits of detection of the RDT compared to culture, where even a few viable cells can be cultured (Alam et al., 2010; Bhuiyan et al., 2003). Discrepancies between the test results were acute when the culture negatives were compared with RDT results, where 48 stool specimens that were negative by culture were positive by RDT lowering the specificity of the rapid test.

It has been recently demonstrated that culture-based method may be underestimating cholera cases in Bangladesh as these methods failed to detect V. cholerae in almost 35% of suspected cholera cases as compared to three different methods including the Crystal VC RDT, a molecular assay, and a direct fluorescent method (Alam et al., 2010). Alam et al. (2010) also postulated that culture negativity may be a result of phage elimination of V. cholerae from the guts of infected people (Faruque et al., 2005). While it is tempting to speculate that in this study, the “false” positives may be “true” positives that may be identified by more sensitive testing methods, the current study was not designed for such rigorous comparative analyses, and more studies must be performed to confirm this finding. To address the problem of comparing RDTs with a less than perfect gold standard, Page et al. (2012) analyzed the results from the Crystal VC RDT using

---

### Table 1

<table>
<thead>
<tr>
<th>RDT</th>
<th>Culture Negative</th>
<th>Culture Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>196</td>
<td>19</td>
<td>215</td>
</tr>
<tr>
<td>Positive</td>
<td>48</td>
<td>381</td>
<td>429</td>
</tr>
<tr>
<td>Total</td>
<td>244</td>
<td>400</td>
<td>644</td>
</tr>
</tbody>
</table>

Sensitivity: 95.3%; specificity: 80.3%.
Positive predictive value: 88.9%; negative predictive value: 91.2%.
statistical approaches using latent class model using Bayesian inferences and a PCR-based methodology. Results of their study demonstrated an increase in sensitivity for RDT and an overall better performance of the dipstick assay (Page et al., 2012).

There are RDTs other than Crystal VC that are commercially available for cholera diagnostics and have sensitivity and specificity, as follows: SMART™ test: 83% and 88%; Medicos™ dipstick: 88% and 80%; and IP dipstick: 94.8% and 76% (Kalluri et al., 2006). These RDTs were performed by laboratory technicians on fresh stool samples, and results of these assays were similar to that found for the Crystal VC RDT in the present study. However, the IP dipstick was never available for purchase, and currently, the Medicos dipstick is not available for purchase. The Crystal VC RDT has been utilized in testing specimens collected as swabs and transported in CB medium; however, this requires an additional 4-hour enrichment step before testing with RDT (Bhuiyan et al., 2003).

When choosing an RDT, relevant operational characteristics including the time required performing and reading the test, ease of use, stability of the test at various storage temperatures, the requirement for additional reagents not included in the test kit, and the feasibility of procurement from the manufacturers should all be considered. In this case, the Crystal VC RDT was easy to set up and read and had good stability. Most of the test kits were stored at ambient temperature for Haiti and were found to be optimal for use when tested for quality assurance. Each testing laboratory should gather quality control (QC) data on tests stored at ambient temperature and at 4 °C over the period of use. The QC data to be collected should include the validity of the internal control and the results obtained from dilutions of both positive and negative controls. Positive (e.g., V. cholerae O1 LIP) and negative control samples should be available to each testing laboratory.

It is important to note that the RDT assay is not recommended for use in individual patient diagnostics; rather, the platform should be positioned as a first-line screening tool and is best suited for outbreak detection. Additionally, the RDT does have discrepancies in performance characteristics when compared to the gold standard i.e., culture-based methods and these differences should be better understood. Finally, the test does need to be stockpiled given the limitations in ordering/receiving large number of Crystal VC kits from the manufacturer; at the same time, consideration should be given to the 12-month expiration date of these kits.

In conclusion, this study demonstrated that the Crystal VC cholera RDT assay was easy to use and interpret in an acute outbreak setting in Haiti with good sensitivity and moderate specificity when compared to the ‘gold standard’ culture-based method. Importantly, the RDT is a field-ready point-of-care test that can have utility in resource-limited situations and settings of continuing outbreaks.

References