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# Evaluation of the SD Bioline Cholera Rapid Diagnostic Test During the 2016 Cholera Outbreak in Lusaka, Zambia

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**Abstract**

**Objective**: To assess the performance of the SD Bioline Cholera Ag O1/O139 rapid diagnostic test (RDT) compared to a reference standard combining culture and PCR for the diagnosis of cholera cases during an outbreak.

**Methods**: RDT and bacterial culture were performed on site using fresh stools collected from cholera suspected cases, and from stools enriched in alkaline peptone water. Dried stool samples on filter paper were tested for *V. cholerae* by PCR in Lusaka (as part of a laboratory technology transfer project) and at a reference laboratory in Paris, France. A sample was considered positive for cholera by the reference standard if any of the culture or PCR tests was positive for *V. cholerae* O1 or O139.

**Results**: Among the 170 samples tested with SD Bioline and compared to the reference standard, the RDT showed a sensitivity of 90.9% (95% CI: 81.3-96.6) and specificity of 95.0% (95% CI: 89.1-98.4).

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After enrichment, the sensitivity was 95.5% (95% CI: 87.3-99.1) and specificity 100% (5% CI: 96.5-100).

**Conclusion**: The observed sensitivity and specificity were within recommendations set by the Global Task Force for Cholera Control on the use of cholera RDT (sensitivity=90% : specificity=85%). Although the sample size was small, our findings suggest that the SD Bioline RDT could be used in the field to rapidly alert public health officials to the likely presence of cholera cases when an outbreak is suspected.

**Keywords:** cholera; rapid diagnostic test; diagnostic accuracy; outbreak

**Introduction**

Cholera is an important public health problem globally. More than 1.3 billion people are at risk, of whom 1.3 to 4.0 million contract cholera with an estimated 21,000 to 143,000 fatalities each year (1). Most of the ~150,000 cases reported each year to WHO are from Africa, Hispanolia and Asia, mainly due to lack of access to safe water, adequate sanitation and poor hygienic practices (2,3). However, these figures are considered to be an underestimate for various reasons such as fear of impact on trade and tourism, shortcomings of surveillance systems, and inadequate timely detection capabilities (2).

Cholera is caused by toxigenic strains of *Vibrio cholerae* O1 and O139 bacteria. Identification of these bacteria in stool by culture or Polymerase Chain Reaction (PCR) is considered the gold standard for cholera diagnosis (4,5). However, both these methods require good laboratory infrastructure and highly skilled staff that are often not readily available in areas where outbreaks occur. Shipment to reference a laboratory and a relatively long turn-around time for culture results, delay the confirmation of cholera diagnosis and implementation of response measures.

Rapid diagnostic tests (RDT) would have great value for the early detection of cholera outbreaks as tools for initial alert and for monitoring of outbreaks or seasonal peaks in endemic areas (6). The use of RDTs could also help prioritize response to the disease in the most affected areas during large outbreaks. Although several immunochromatographic RDTs are commercially available, most of the recent diagnostic evaluations have focused on one product, Crystal VC (Arkray Health Care Private Limited, India; previously Span Diagnostics, India), which has shown high sensitivity of more than 90%, but moderate specificity (49-88%) when used directly on stools (7–11). In addition, relatively high proportions of false-positive results with the O139 line have been reported by several teams (12–14). The specificity of this test can be improved by a four to six-hour enrichment step in alkaline peptone water (APW), with the disadvantage of increasing the necessary logistics required and delaying the turn-around time for results to be available (13,15,16). First data on two other tests,

Artron *Vibrio cholerae* O139 and O1 Combo Test (Artron Laboratories Inc, Canada) and SD Bioline Cholera Ag O1/O139 RDT (Standard Diagnostics Inc., Korea), recently came from a retrospective analysis of routine data in Haiti comparing results of cholera RDTs to culture results (12). Whereas the Artron test, like Crystal VC, showed very high sensitivity of 98.6% and modest specificity around 70%, the SD Bioline RDT had a lower sensitivity of 81.1% but specificity of 92.8%.

In a cholera outbreak that occurred in Lusaka, Zambia, from February to June 2016, the Ministry of Health (MoH) of Zambia implemented an enhanced cholera surveillance system early in the course of the outbreak using the SD Bioline Cholera Ag O1/O139 rapid test. Following an emergency reactive oral cholera vaccine (OCV) campaign, organized in April 2016 by the MoH, in collaboration with Médecins Sans Frontières, a study was implemented to estimate the vaccine effectiveness conferred by one dose of OCV (17). Stool specimens collected from suspected cholera cases were sent to the laboratory for confirmation by culture and PCR, in addition to the RDT. We took advantage of this study to evaluate the diagnostic performance of SD Bioline RDT, aiming to identify an effective screening tool for cholera in an emergency situation or outbreak.

## Methods

### *Ethics*

This was a sub-study of the main study titled "Effectiveness of one dose of killed whole cell cholera vaccine in response to an outbreak". The protocol for the main study was approved by the Ethical Review Boards of the University of Lusaka (Zambia) and the Johns Hopkins School of Public Health (USA). Written informed consent was obtained from participants or their parents/guardians. Privacy and confidentiality of the data collected from participants was ensured both during and after the study.

### *Study site and Population*

This study was conducted in Lusaka district, Zambia, which has a population of approximately 2.3 million. The outbreak started on February 4th, 2016 and lasted until June 15$^{th}$, 2016, and the MoH reported 1139 confirmed and/or suspected cholera cases and 20 cholera-related deaths (18).

Two cholera treatment centers located in Kanyama and Bauleni and three cholera treatment units located in Chawama, Matero and George served as study sites. These clinics provided access to treatment to all patients coming from the high-risk areas in Lusaka. As part of enhanced surveillance, all suspected cholera cases (patients with acute non-bloody watery diarrhea with more than three liquid stools in 24 hours) gave a stool sample that was sent to Kanyama clinic laboratory for microbiological testing. The MoH enhanced capacity to carry out microbiological tests for detection

and confirmation of cholera at Kanyama clinic laboratory by seconding a microbiologist from the National Reference Laboratory of the University Teaching Hospital (UTH).

Stool samples from patients with acute non-bloody diarrhea who presented from April 25th to June 15th 2016 to one of the treatment centers participating in the vaccine-effectiveness study and enrolled according to inclusion criteria (17) were included in the study. We excluded from this analysis patients enrolled after June 3rd, for whom SD Bioline rapid diagnostic testing could not be carried out because of a stock out of available kits.

***Laboratory procedures***

*Specimen Collection and Preparation*

Fresh stool samples were collected in a clean unchlorinated disposable container. The samples were immediately transported at room temperature (from Kanyama clinic) or in a cool box (from other study sites) to Kanyama clinic laboratory for testing.

Once the stool sample arrived in the laboratory, laboratory technologists performed cholera RDT and culture (see details below) directly from the sample. They also inoculated two drops in APW, which was incubated for 4 to 6 hours at 37°C. The RDT and culture were then repeated on the sample enriched in APW. Additionally, laboratory technologists placed two drops (approximately 80-100 µL) of undiluted sample on Whatman 903 Protein Saver Card (GE Healthcare Ltd, Forest Farm, Cardiff UK), which was stored for further testing by PCR.

*Rapid Diagnostic Test Procedure*

The testing was performed by qualified and trained laboratory technologists at Kanyama clinic laboratory and supervised by a microbiologist from the National Reference Laboratory. Technologists performing the rapid tests were blinded to clinical information and to the results of culture and PCR, which were performed subsequently.

SD Bioline Cholera Ag O1/O139 was used for testing on 170 samples following manufacturer's instructions when performing the tests and interpretation of results. Briefly, the stool sample was collected using a cotton swab, which was then swirled in a sample collection tube pre-filled with 1 mL of sample diluent buffer. Three to four drops of diluted sample were then added into the sample well of the test device using the cap with dropper nozzle of the collection tube. The test was read after 10-20 minutes and interpreted as negative if only the control line appeared, positive for O1, O139 or both if the control line and the corresponding line(s) appeared, and invalid if the control line did not appear .

*Stool Culture*

Stool samples were streaked out on Thiosulphate Citrate Bile Sucrose Agar (TCBS; Oxoid, UK) directly and after 4-6 hours of incubation in APW. After 18-24 hours incubation at 37°C, TCBS plates were examined for the presence of yellow colonies suggestive of *V. cholerae.* Single well isolated yellow colonies were picked and streaked on Mueller-Hinton agar (Oxoid, UK) and incubated at 37°C for 24 hours. Colonies on Mueller Hinton agar were tested for oxidase and, in case of those positive for the oxidase reaction, testing with O1 polyvalent, O1 Inaba, O1 Ogawa and O139 antisera (Beckton Dickinson, USA) was performed as previously described (19).

*PCR Analysis*

PCR was performed using stool samples stored on Whatman 903 Protein Saver Card kept at ambient temperature following previously described methods (13). Total DNA extraction was performed by thermal shock method. Briefly, one circle containing dried stool was placed in a microtube and rehydrated with 150 µL sterile water. Two hundred microlitres of 2% Chelex solution was then added and the sample was vortexed at high speed, boiled for 8 minutes and centrifuged for 2 minutes (20).

Polymerase-chain reaction (PCR) was performed at Institut Pasteur, Paris, and in parallel at the UTH laboratory following same protocols as part of technology and methodology transfer. PCR was performed on 2 µL of the supernatant to detect an intergenic spacer region specific of *V. cholerae* species using primer sequences (F-TTA AGC STT TTC RCT GAG AAT G and R-AGT CAC TTA ACC ATA CAA CCC G) (21). On samples positive for *V. cholerae*, the *rfb* gene was amplified for the identification of *V. cholerae* serogroups O1 and O139, as described by Hoshino (22). On negative samples, the PCR was repeated on 4 µL volumes. If still negative, a 16S rRNA PCR was performed on the samples to assess the presence of DNA and/or PCR inhibitors as described previously (13). DNA from known *V. cholerae* O1/O139 strains supplied by Pasteur Institute, France were used as positive controls.

The technologists performing PCR were blinded to the RDT results and clinical information, but were aware of culture results.

***Statistical analysis***

For the reference standard, a true cholera case was defined as a suspected case with at least one culture or PCR positive for *V. cholerae* O1. A true negative case was defined as a suspected case with all culture and PCR results negative for *V. cholerae* O1. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were estimated for both direct testing and testing after 4-6 hours of enrichment in APW, by comparing the RDT results to the reference standard

defined above. The estimates and 95% confidence intervals for sensitivity, specificity, PPV, and NPV were estimated in Stata SE 14 (Stata Corporation, College Station, Texas) using the exact binomial method. The kappa coefficient was calculated to estimate the overall concordance between the rapid test and gold standard.

**Results**

From April 25th to June 15th, 2016, 251 patients with acute non-bloody diarrhea were admitted and treated at health centers in the study area and 211/251 (84%) patients were recruited in the parent vaccine effectiveness study during that period. Of these, 170/211 (81%) had their stool samples tested with SD Bioline from April 25th to June 5th and were included in this evaluation (Figure 1). The majority of stool samples tested were from participants above 15 years of age and those who were severely dehydrated (Table 1).

***Culture and PCR results***

Of the 170 samples tested with SD Bioline, 62 (36.4%) samples were positive for *V. cholerae* O1 Ogawa by culture while 63 (37.1%) samples were positive for *V. cholerae* O1 by PCR at the University Teaching Hospital (UTH), Lusaka, and 62 (36.4%) samples positive for *V. cholerae* O1 by PCR at Institut Pasteur (IP), Paris. Three culture-negative specimens were detected by both PCR series, while 3 culture-positive specimens were missed by each of the PCR series. Finally, 66 patients (38.8%) were considered positive for cholera by the reference standard and 104 (61.2%) negative.

***Rapid diagnostic test performance***

Of the 170 samples tested with SD Bioline, 65 were positive for *V.cholerae* O1 by the RDT performed directly on stool and 63 by the RDT performed on APW-enriched sample (Table 2). None of the RDTs showed a positive O139 line. The overall agreement between the RDT and the gold standard was kappa=0.86 for the direct test and kappa=0.96 after enrichment. The SD Bioline RDT had a sensitivity of 90.9% and specificity of 95.2% when performed directly on the stool sample, which increased to 95.5% and 100%, respectively, after enrichment (Table 2). The performance estimates remained similar when analysis was restricted to patients without prior antibiotic consumption, which is sometimes considered an exclusion criteria in diagnostic evaluations (Table 2).

**Discussion**

Our evaluation indicates that the SD Bioline Cholera Ag O1/O139 rapid test is promising, in terms of both sensitivity and specificity, for the diagnosis of cholera. With estimates of sensitivity at 90% and

specificity at 95%, the SD Bioline RDT matches the recommendations of minimal performance of 90% sensitivity and 85% specificity set forth in the Interim Technical Note on the use of cholera RDT published by the Global Task Force for Cholera Control (GTFCC) (6). This test also matches the recommendations from a recent Target Product Profile for cholera RDTs recently developed by the GTFCC (23), in terms of cost (~2€ per test), ease of use, and clinical performance, although the confidence intervals are too wide due to the relatively small sample size in our study. This, together with the recent retrospective analysis of routine results in Haiti showing a more modest sensitivity (81.1%) of the SD Bioline RDT compared to culture (12), highlight the need for additional prospective results on larger sample sizes and in different contexts.

The performance of the SD Bioline test reported here and in Haiti (12) suggest that the specificity of SD Bioline might be better than that of Crystal VC (49-88%), but that its sensitivity might be lower than Crystal VC (consistently around 92-97%) (7–11). High sensitivity is generally considered the most important criterion for screening tests, while moderate specificity can be overcome by re-testing initially reactive samples using more specific confirmation assays. In the case of cholera, positive RDT results are currently considered as a cholera alert, which should prompt the shipment of samples to a central laboratory for confirmation by culture or PCR (6). However, this confirmation step is sometimes challenging or not available, and further delays the implementation of response measures. The availability of a rapid test with high specificity might change the paradigm, so that positive RDT results could be considered as highly indicative of cholera, and targeted response measures can be started immediately. In this case, high specificity would be crucial, while moderate sensitivity could be overcome by testing a sufficient number of samples from suspected cholera cases to ensure that cholera is detected, as has historically been done with culture. Indeed, although considered the gold standard for cholera diagnosis, culture does not have perfect sensitivity (24). In a recent study in South Sudan, the sensitivity of culture compared to PCR was estimated at 83% when performed on-site and 72% for a delayed culture at an international reference laboratory, which was lower than the sensitivity of SD Bioline estimated here (13).

In our study, the same number of positive results was detected by culture on site, PCR at UTH and PCR at IP, suggesting that PCR was not much more sensitive than culture in these conditions. In addition, each set of PCR taken individually would have missed three culture-positive results. It should be noted that culture was performed directly on fresh stools, whereas PCR was done later using stool samples stored on dry filter paper. In contrast, in the study mentioned above in South Sudan, both culture and PCR were done at a later stage from dry or wet filter paper (13). This highlights the varying relative performance of culture and PCR depending on the sample collection and storage conditions and the need for a proper comparison of these methods. Whereas PCR could

be more sensitive than culture when performed on the same volume of specimen stored in the same conditions, its added-value compared to good-quality culture on fresh stool might be limited, considering also the possible presence of PCR inhibitors in stools. However, considering the importance of the reference standard in diagnostic evaluations and the impact of an imperfect reference standard on performance estimates (7), the few additional cases identified by PCR do have their importance for proper patient classification. This should be considered when defining the reference standard for future evaluations of cholera rapid tests.

Enrichment in APW for 4-6 hours has been reported to improve the performance of Crystal VC in several studies (13,15,16). Although not recommended by the manufacturer, this enrichment step was also tested here with the SD Bioline RDT and shown to improve performance. The difference was small and, in practice, the added-value in performance might not be worth the added complexity and longer turn-around-time of this method for routine use. However, this method could potentially be very useful in cases where laboratory confirmation is not available and a specificity of 100% would be required.

This study had several limitations. First, the sample size was limited, in particular for cholera-confirmed cases, leading to wide confidence intervals in the estimates. Whereas studies set up in outbreak situations with comprehensive outbreak response measures often have limited sample sizes (11,25), larger sample sizes can be obtained in endemic settings (12). Alternately, performing meta-analyses using data from different studies and settings with similar study methods might be another way forward to get better insights into the performance of these tests. Second, the tests were done by trained laboratory technologists in a study setting, which might limit the possibility to extrapolate these results to more challenging field conditions with less-trained users.

Several factors, including those discussed above, could explain the higher sensitivity of the SD Bioline RDT in our study compared the analysis of routine data collected in Haiti (12). First, it should be noted that due to the limited sample size in our study, the confidence intervals around the sensitivity estimates do overlap. Second, the specimen transfer method using a cotton swab might lack standardization in the volume of specimen actually transferred into the sample collection tube, which could affect sensitivity of the assay. The format of the test kit was recently modified to include a dropper to transfer liquid stools, which should make the volume of specimen used in the assay more reproducible. Third, some characteristics of the study population, such as age, severity of disease, delay between onset and stool collection, or cholera endemicity, could have an impact on the bacterial load in stool specimens, which could in turn influence the clinical sensitivity if the bacterial loads are close to the analytical sensitivity of the assay. Population characteristics were not reported in the routine data from Haiti (12). Finally, the reference standards used in these analyses

were different, as was the type of data collection, prospective versus retrospective.

In conclusion, these data on the performance of the SD Bioline Cholera Ag O1/O139 RDT suggest that this test could be used in the field to launch cholera alerts and maybe even start response measures, considering its good sensitivity (90.9%) and high specificity (95%). However, these results need to be reproduced with larger sample sizes and in different contexts representative of remote settings where cholera RDTs are most needed. Even if these evaluations confirm the good performance of this test, culture and PCR will remain paramount for the characterization of cholera strains and understanding of global epidemiology of cholera in order to better fight this deadly disease and continuing pandemic.

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**Table 1.** Characteristics of the patients included in the study, overall and among those with cholera confirmed or not by the reference standard

| | All (N=170) | Cholera (N=66) | No cholera (N=104) |
|---|---|---|---|
| | n (%) | n (%) | n (%) |
| Female sex | 81 (47.7) | 28 (42.4) | 53 (51.0) |
| Age in years, mean (SD) | 23.9 (18.5) | 22.7 (16.2) | 24.6 (19.9) |
| < 5 | 38 (22.4) | 11 (16.7) | 27 (26.0) |
| 5 - 15 | 21 (12.4) | 11 (16.7) | 10 (9.6) |
| > 15 | 111 (65.3) | 44 (66.7) | 67 (64.4) |
| Dehydration* | | | |
| A (no dehydration) | 11 (6.5) | 0 (0) | 11 (10.6) |
| B (mild) | 45 (26.5) | 7 (10.6) | 38 (36.5) |
| C (severe) | 113 (66.5) | 59 (89.4) | 54 (51.9) |
| Antibiotics | | | |
| within 2 days before admission | 18 (10.6) | 8 (12.1) | 10 (9.6) |
| at CTC before sample collection | 52 (31.0) | 26 (39.4) | 26 (25.0) |
| Received oral cholera vaccine | 26 (15.3) | 6 (9.1) | 28 (26.9) |

*CTC, Cholera Treatment Center; SD, Standard deviation*

**Table 2.** Diagnostic performance of SD Bioline performed as per manufacturer's recommendation (direct) or after enrichment in APW

| | Reference standard | | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|
| | Positive | Negative | % (95% CI) | % (95% CI) | % (95% CI) | % (95% CI) |
| **All (N=170)** | | | | | | |
| Direct | | | 90.9 (81.3-96.6) | 95.2 (89.1-98.4) | 92.3 (83.0-97.5) | 94.3 (88.0-97.9) |
| Positive O1 | 60 | 5 | | | | |
| Negative | 6 | 99 | | | | |
| After enrichment | | | 95.5 (87.3-99.1) | 100 (96.5-100) | 100 (94.3-100) | 97.2 (92.0-99.4) |
| Positive O1 | 63 | 0 | | | | |
| Negative | 3 | 104 | | | | |
| **No prior antibiotics (N=101)** | | | | | | |
| Direct | | | 91.2 (76.3-98.1) | 95.5 (87.5-99.1) | 91.2 (76.3-98.1) | 95.5 (87.5-99.1) |
| Positive O1 | 31 | 3 | | | | |
| Negative | 3 | 64 | | | | |
| After enrichment | | | 94.1 (80.3-99.3) | 100 (94.6-100) | 100 (89.1-100) | 97.1 (89.9-99.6) |
| Positive O1 | 32 | 0 | | | | |
| Negative | 2 | 67 | | | | |

*PPV, positive predictive value; NPV, negative predictive value*

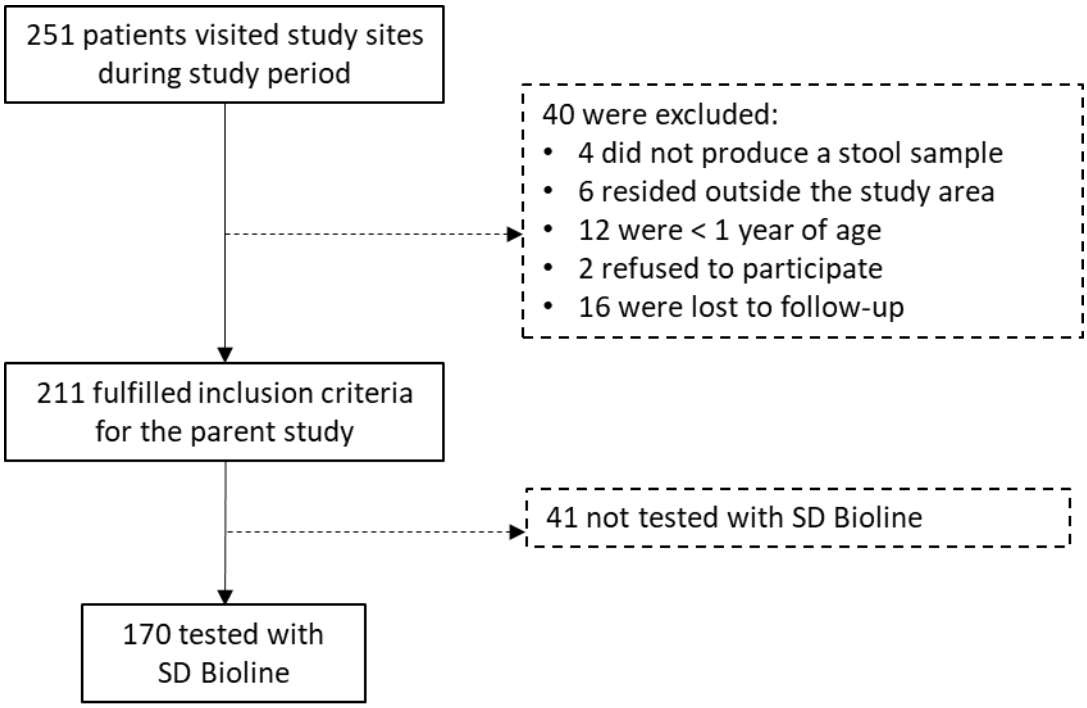


**Figure 1.** Study flow diagram