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Evaluation of Crystal® VC Rapid Diagnostic Test kit to detect *Vibrio cholerae* from fecal samples in Ethiopia

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Abstract

Background: *The Crystal® VC Rapid Diagnostic Test (RDT) has been shown to be a sensitive, rapid, cost-effective, and time-efficient tool for detecting V. cholerae. Although this test has advantages in terms of sensitivity and specificity, there are no reports on how well it performs across different regions and during various seasons in Ethiopia to assess its robustness.*

Objective: *The study aimed to evaluate the diagnostic performance of Crystal® VC RDT rapid Diagnostic Test to detect V. cholerae from fecal samples in Ethiopia.*

Method: *A cross-sectional study was conducted from October 2022 to February 2024 at cholera outbreak sites across Ethiopia. A total of 361 fecal samples were collected and tested concurrently using Crystal® VC RDT and standard culture methods. Sensitivity, specificity and positive and negative predictive value of the Crystal® VC RDT were calculated using culture as the gold standard. Agreement between the two diagnostic methods was assessed using Cohen's Kappa statistic. Data analysis was performed using SPSS version 25 and MedCalc computer software.*

Results: *Of the 361 fecal samples analyzed, 123 (34%) were confirmed positive for Vibrio cholerae by culture. The Crystal® VC RDT demonstrated a sensitivity of 98.4% (95% CI: 94.3% –99.8%) and a specificity of 52.1% (95% CI: 45.6%–58.6%).The PPV was 51.5% (95% CI: 48.1%–54.8%) and the NPV was 98.4% (95% CI: 94.0%–99.6%). The test showed a moderate agreement with culture (kappa =0.679).*

Conclusion: *Crystal® VC RDT demonstrate high sensitivity and excellent NPV, making it a valuable tool for early detection and rapid response during cholera outbreaks. Despite lower specificity, the test's speed and ease use support its utility in field settings and epidemiological surveillance in resource-limited areas.*

Introduction

Cholera is an acute bacterial gastrointestinal non-invasive disease that affects many low- and middle-income countries (1). Individuals diagnosed with cholera exhibit acute watery diarrhoea, experiencing three or more episodes within a 24-hour period (2). Cholera is caused by *Vibrio cholerae* (*V. cholerae*) which is a Gram-negative bacteria (3). Cholera outbreak is a major public health emergency disease, it results high socioeconomic disruption, morbidity and mortality across the world (4, 5). Currently, there are an estimate of 1.3-4.0 million cholera cases and 21,000-143,000 cholera annual deaths in the world. Cholera remains a global threat to public health which is also an indicator of inequity and lack of social development (6, 7). The global burden of cholera is high in all age groups. However, the incidence and mortality increased in children under five years old (8).

In Ethiopia, cholera impacts 70 million people each year, with an estimated 275,221 cases and 10,458 deaths annually (9). As Office for the Coordination of Humanitarian Affairs (OCHA) report, about 29,800 cholera cases and more than 400 deaths were reported across 10 regions of Ethiopia between January 2023 and January 2024. According to Access Capacities Project (ACAPS) thematic cholera report in Ethiopia, conducted on 18th January 2024, the highest number of cases were reported in Oromia National Regional State, Amhara National Regional State, and Somali National Regional State, respectively (10).

The identification of the causative agents of epidemic cholera requires microbiological diagnostic methods, such as culture, PCR, and RDT (11). Culture techniques can be utilized to grow, isolate, and characterize *V. cholerae* (12). Culture is gold standard method for detecting *V. cholerae*. This is then followed by biochemical identification and serotyping using both polyclonal and monoclonal antibodies (13). However, PCR offers greater accuracy and sensitivity for *V. cholerae* detection and is increasingly adopted in diagnostic laboratories for timely and reliable results (14). In resource limited settings, culture and PCR methods for detecting *V. cholerae* are often impractical due to their lengthy processing times (at least 18 hours for culture), the need for highly trained personnel, high costs, and the requirement for specialized laboratory infrastructure (15). Nowadays, Crystal® VC RDT has been developed and is accessible for detecting the causative agents of cholera from clinical samples (16). The new version of Crystal® VC RDT kit was manufactured by Arkray Healthcare Pvt. ltd, at Gujarat, India (17). Crystal® VC RDT was designed to detect *V. cholerae* O1 and O139 in fecal sample using a rapid visual immunochromatographic assay, and has sensitivity range 88-100% and specificity 61-87.3% (18, 19). Crystal® VC RDT is easy to use and can be used as a portable device from one location to another (20). In addition, Crystal® VC RDT is utilized at point-of-care facilities to enhance decision-making in the timely management of cholera outbreaks and to investigate the epidemiology of the disease, particularly during surveillance activities. This helps to minimize the spread of outbreaks and reduce mortality (21). As far as we know, there are no reports on how well the recently introduced Crystal® VC RDT test performed in Ethiopia. Therefore, this study aimed to evaluate the performance of Crystal® VC RDT for detecting *V. cholerae* from diarrhoea samples in Ethiopia.

Method

Study area, design and participants

A cross-sectional study was carried out from October 2022 to January 2024 at outbreak sites in the three regional states in Ethiopia. Fecal samples were collected from each participants suspected of cholera and attending treatment at the different outbreak sites in Oromia National Regional State (Bale zone, Guji zone, west Arsi and Madda Walabu), Amhara National Regional State (West Gondar zone, Bahir Dar Zuria and Awi zone) and Addis Ababa City Administration (Kolfe Qeranio sub-city).

Sample size: The sample size was determined using the single population proportion formula.

$$n=Z^2 * p(1-p)/E^2$$

p= population proportion=50%

z= z-score=1.96

E = margin of error=5%

n= study sample size

$$n= (1.96)^2 * 0.5(0.5)/(0.05)^2 = 384$$

First a total of 384 individuals suspected of cholera were enrolled. Twenty-three participants were excluded from the study due to mislabeling or delays in collection of their fecal samples. The final sample size was 361. Of these, the majority of the study participants were collected from Oromia Na-

tional Regional State (223), from Amhara National Regional State (73), from Addis Ababa City Administration (65). The study participants were selected using convenient sampling technique.

Inclusion and exclusion criteria

Participants who were admitted to the cholera treatment center (CTC) at each outbreak site due to acute watery diarrhoea were included. However, participants who had been on antimicrobial treatment for the past two weeks before sample collection were excluded from this study.

Fecal sample collection, storage and transportation

According to the national cholera sample collection protocol, two aliquot of fecal samples were collected from each patient suspected of cholera disease (22). The fecal samples were collected by using wide mouthed, leak proof, clean, and dry container. One of the aliquot of the fecal sample was used for detection of *V. cholerae* with Crystal® VC RDT (Arkray Healthcare Pvt. ltd, at Gujarat, India) at the CTC and the other aliquot of the fecal sample was used for culture to isolate *V. cholerae* at the microbiology diagnostic laboratories.

Crystal® VC Rapid Diagnostic Test

Aliquot of fecal samples were immediately tested using the Crystal® VC RDT to detect the presence of *V. cholerae* O1 and/or O139 at the CTC. Briefly, 200µl of fresh watery fecal samples were taken by a pasture pipette and dispensed directly to the well of the Crystal® VC RDT test kit. The preparation was allowed to stand for 15 - 30 minute at room temperature until the test and the control line are visible and removed after 30 minutes. The Crystal® VC RDT results were observed and the results determined as positive or negative by two medical laboratory science professionals. A positive result appears as two or three pink lines on the kit, the one being the control band and the other line being the band specific to either serogroup O1, or serogroup O139 or both serogroups. The results were recorded and interpreted according to the manufacturer's protocol. If the control line did not appear visible regardless of the test lines, the test was considered invalid and repeated once (21).

Detection of *V. cholerae* using Culture

The second aliquot of fecal samples were placed into Kari Blair transport media and kept cold using an ice pack and then transported to the nearby microbiology laboratory available sites to Shashemene General Hospital, Armauer Hansen Research Institute and Amhara Public Health Institute for sample processing and laboratory analysis. Fecal samples were inoculated onto Blood Agar (BAP, Oxoid), MacConkey Agar (MAC, Oxoid), and Thiosulfate Citrate Bile Salt Sucrose (TCBS, Oxoid) agar, and then incubated at 37°C for 24 hours. Identification of *V. cholerae* was performed using a series of biochemical tests, including the Oxidase test, String test, Motility test, Indole test, Citrate test, Gas production test, H2S production test, and Urease test, all from Oxoid. Polyvalent and monovalent antisera (manufactured by Deben Diagnostics Ltd, USA) were utilized for agglutination tests to identify and differentiate *V. cholerae* serogroups and serotypes. The culture method was performed independently of the Crystal® VC RDT results to ensure unbiased outcomes.

Quality control

Data collected daily was recorded and compiled. A laboratory protocol was prepared and strictly followed. The principal investigator was responsible for monitoring all steps of data collection and recording. The reagents were checked for expiry date and appropriate storage temperature and humidity. In parallel, both positive and negative controls were included. *Vibrio cholerae* reference strains; N16961 or C6706 (O1 El Tor) and MO45 (O139) were used as controls based on the combination of conventional biotyping methods. Quality assurance was ensured with good practice in preparing and reading.

Data Analysis

All data were coded and checked to detect an error and transferred from a questionnaire to excel, and then to SPSS version 27. The definition conventional culture was used as gold standard and considered as a reference for Crystal® VC RDT. The sensitivity, specificity, predictive value, prevalence and accuracy were analyzed by SPSS version 25, and MedCalc statistical software. The kappa (k) statistics and ROC curve were calculated by SPSS and used to compare the agreement between the RDT result and the Gold standard culture result. The interpretation of the agreement was as follows no agreement if k value < 0, poor agreement if k value = 0 – 0.2, fair agreement if k value = 0.21 – 0.4, moderate agreement if k value= 0.41-0.6, substantial agreement if k value= 0.61- 0.8 and excellent agreement if k value > 0.8 (23).

Ethical considerations

Ethical clearance was obtained from the University of Gondar Institutional Review Board (IRB) (R. No. *VP/*

RTT/05/20/2022). Written informed consent was obtained from participants after explaining the purpose and objective of the study. In addition, formal written consent was obtained from the parent/guardian. Participants had a full right to continue or withdraw from the study. All information was kept confidential by assigning code and assessed only by the principal investigator and supervisors. The laboratory results were communicated with concerned stakeholders and participants. If the fecal sample was positive either one or both methods for *V. cholerae*, we communicated with concerned health professionals, and treated patients according to WHO cholera outbreak management guidelines.

Result

A total of 361 participates suspected of cholera were included and fecal samples were collected. The data indicated that 54.9% of the study participants were female, and 52.8% participants were living in rural areas. The average age of participants was 23 years, with a range covering from 1 to 80 years. Nearly half (47.2%) of the participants were aged between 6 and 18 years **(Table 1).**

Table 1: Demographic characteristics of participants involved in cholera outbreak study in Ethiopia.

Variables	Category	Frequency	Crystal® VC RDT positive (%)	Culture positive (%)
Age	1-5	85	46 (54.1)	10 (11.8)
	6-18	166	118 (70.1)	65 (39.2)
	≥19	110	71 (64.5)	48 (43.6)
Sex	Male	164	112 (68.3)	59 (36)
	Female	197	123 (62.4)	64 (32.5)
Region	Oromia	225	125 (55.6)	34 (15.1)
	Amhara	71	67 (94.4)	59 (83.1)
	Addis Ababa	65	43 (66.2)	30 (46.2)
Residence	Urban	172	111 (64.5)	55 (32)
	Rural	189	124 (65.6)	68 (36)
Educational status	Can't read and write	145	88 (60.7)	31 (21.4)
	Elementary	189	129 (68.3)	77 (40.7)
	Secondary	4	4 (100)	4 (100)
	Higher	23	14 (60.9)	11 (47.8)
Marital status	Married	160	113 (70.6)	79 (69.9)
	Unmarried	186	115 (61.8)	38 (20.4)
	Divorced	11	6 (54.5)	5 (45.5)
	Widowed	4	1 (25)	1 (25)
Frequency of diarrhea	1-3	34	24 (70.6)	11 (32.4)
	≥3	327	211 (64.5)	112 (34.3)

Crystal® VC RDT: Crystal *Vibrio Cholerae* Rapid Diagnostic Test

Evaluation of Crystal® VC RDT to detect *Vibrio cholerae* compared with culture

The study showed that (65.1%, n=235) of fecal samples were found positive for *V. cholerae* by using the Crystal® VC RDT, while (34.1%, n=123) of fecal samples were detected positive for *V. cholerae* by using the culture method. On the other hand (34.9%, n=126) of the samples were negative for *V. cholerae* by both methods (Table 2).

In the present study, the Crystal® VC RDT demonstrated the following performance metrics: sensitivity, specificity, positive and negative predictive value were revealed as follows (Table 3).

Table 2: Comparison of the e Crystal® VC RDT and the conventional culture method.

Test methods		Culture (N=361)		Total
		Positive	negative	
Crystal® VC RDT (N=361)	positive	121	114	235
	negative	2	124	126
Total		**123**	**238**	**361**

N: Number

Table 3: Diagnostic performance of the Crystal® VC RDT test compared with the gold standard culture method to detect *V. cholerae* from fecal samples.

Parameters	Performance (%)	95%CI
Sensitivity	98.4	94.3 - 99.8
Specificity	52.1	45.6 – 58.6
Positive predictive value	51.5	48.1 -54.8
Negative predictive value	98.4	94.0- 99.6
Prevalence	34.1	29.3 – 39.2

CI: confidence interval

Using the culture method as gold standard for detection of *V. cholerae*, the kappa agreement of the Crystal® VC RDT was 67.9% (p=0.001).

Receiver operating characteristic curve (ROC) curve analysis

Moreover, the overall accuracy of the Crystal® VC RDT was deemed acceptable with an area under the ROC curve was determined 67.9% (95%CI: 62.8% – 72.7%) (**Figure 1).**

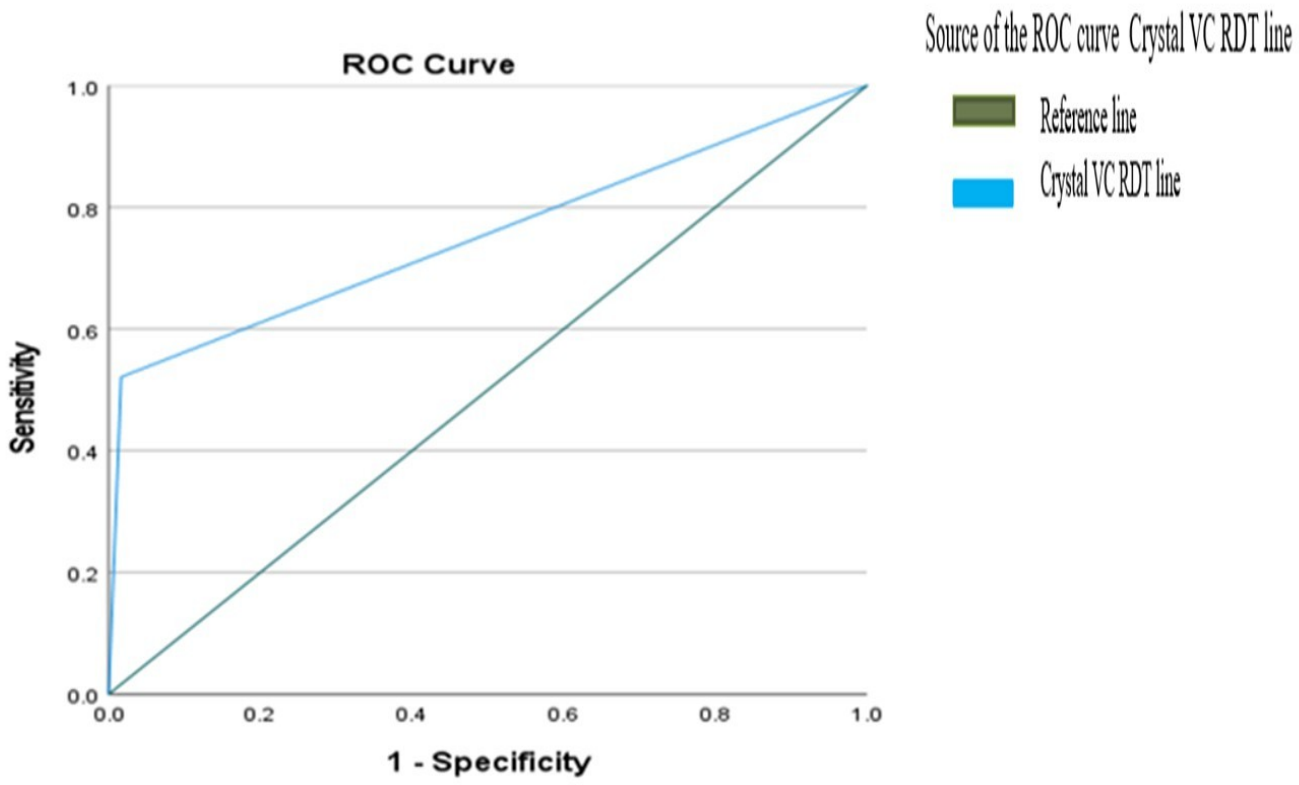

Figure 1. Receiver operating characteristic (ROC) curve for Crystal® VC RDT kit. The X-axis represents false positive rate (1-specificity) and the Y-axis represents true positive rate (sensitivity). The diagonal green line represents random classification (reference line. Thus, the ROC curve is a plot of tests sensitive versus 1-specificity as well. The closer this curve is to the upper left corner, the better the diagnostic significance.

Discussion

Crystal® VC RDT kit has been marketed as an alternative to the conventional culture method, particularly in high cholera outbreak attacked countries having resource-limited laboratory settings (17). The current study assessed the significance, and performance of the Crystal® VC RDT kit in comparison to the conventional culture method, which is considered as the gold standard for *V. cholerae* diagnosis.

In this study, the sensitivity of the Crystal® VC RDT was 98.4% (95%CI; 94.3% - 99.8%). Previously, Chowdhury et al. from India showed exactly a similar report (98.4%) of sensitivity of Crystal® VC RDT compared with gold standard culture (24), and another report from the Democratic Republic of Congo demonstrated lower sensitivity (92.2%) (16), and in Hati, Lower sensitivity (71.2%) and lower positive predictive value (81.3%) were previously reported (25). The present study findings were greater than the previous WHO sensitivity, specificity, positive predictive value, negative predictive value reports of 91.3%, 43.1%, 72.8%, 74.8%, respectively (26). This observed discrepancy may be attributable to several methodological and contextual variables, such as sample quality, differences in the technical proficiency and experience of healthcare personnel, and heterogeneity in the types of biological specimens analyzed. Each of these factors has the potential to influence diagnostic accuracy of the current and previous WHO investigations (27).

The specificity of the Crystal® VC RDT was relatively lower 52.1% (95%CI; 45.6% – 58.6%). The study was comparable with Ley B. et al. specificity report in Zanzibar (49.2%) (19). Data of the present study on the positive and negative predictive values for the Crystal® VC RDT were 51.5% and 98.4%, respectively. This study findings were aligned with the manufacturer's report range for the Crystal® VC RDT, which is 88-100% for sensitivity and 61-87.3% for specificity (17, 24). Collective evidence shows that Crystal® VC RDT is a test with high sensitivity, high negative predictive value, and low specificity, with lower positive predictive values.

In the present study, the Crystal® VC RDT had a moderate Kappa agreement of 67.9% (p=0.001) with the gold standard culture. The kappa agreement of our study was less than the kappa agreement of 98.06% a study conducted in Kenya (28). In addition, Crystal® VC RDT had moderate accuracy under the ROC curve analysis of 67.9% (95%CI= 62.8%–72.7%). Crystal® VC RDT does not require exclusive equipment and facilities which makes it easy and applicable in resource limited settings like Ethiopia. In general, the findings indicate that the Crystal® VC RDT showed moderate agreement with the gold standard culture method, affirming its reliability to use as a *V. cholerae* detection tool during cholera outbreak in resource limited settings and health institutions (21).

Having higher sensitivity to Crystal® VC RDT is very essential, especially during the occurrence of cholera outbreak (29). Because a highly sensitive diagnostic test increases the detection rate of the causative agents of the cholera outbreak (20). This helps to detect *V. cholerae* rapidly among infected patients and will be helpful in providing immediate patient management (25). The use of Crystal® VC RDT is more essential at the point-of-care facilities as it helps to make appropriate decisions in the management of outbreaks or epidemiological surveillance by the public health authorities (30). Crystal® VC RDT is simple easy to use, fast, cheap and can be stored without refrigeration (13, 31). Crystal® VC RDT is used to detect lipopolysaccharide antigens from *V. cholerae* O1 and/or O139 serogroups in fecal samples, which are also present in oral cholera vaccines (32). Hence, Crystal® VC RDT test could be used as a point of care test (POCT) detection tool to *V. cholerae* and used to detect vaccine efficacy within one week after vaccine providing (33).

Furthermore, this Crystal® VC RDT also helps to the Global Task Force on Cholera Control's (GTFCC) roadmap/ program to End Cholera by 2030, because the Crystal® VC RDT is used for rapid detection of *V. cholerae* in resource limited settings, and in health institutions (34). One of the limitations of the Crystal® VC RDT is that some results are demonstrated with faint test lines that might be observed as positive results which is one of the serious limitations of the test. In addition, the presence of lipopolysaccharide antigen in *V. cholerae* and other Gram-negative bacterial species contributes to false positive readings in the Crystal® VC RDT (17). However, it has been promising strength due to its sensitivity, rapid detection of the outbreak, user friendly and low cost in frequently cholera outbreak affected countries (35).

Conclusion and recommendations: The sensitivity and negative predictive value of the Crystal® VC RDT was high. Crystal® VC RDT is a user-friendly, rapid, equipment free option to use as a POCT. The use of Crystal® VC RDT for detection of *V. cholerae* in the facility and in field settings is therefore beneficial due to its comparative advantage over the culture method for being sensitive, low cost, and easy to use. In addition, this test uses for early detection of *V. cholerae* outbreak and epidemiological surveillance. Furthermore, the test uses to detect vaccine efficacy within one week. Therefore, our study suggests that to use Crystal® VC RDT for the preliminary detection of cholera during the occurrence of the outbreak. Furthermore, health professionals are recommended to use Crystal® VC RDT to detect cholera in order to provide immediate patient management.

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Author's contribution: AB, AG, YW and BG conception, design and analysis of the study; A.B collected the data and performed laboratory investigation, statistical analysis, interpretation of the results and manuscript writing. BG, AG and YW involved in manuscript draft writing, statistical analysis, approved quality of the data and interpretation of the results. BY, MT, AA, GY, GT, MB, TB, MA, and AM contributed in the manuscript writing, statistical analysis and interpretation of the results. All authors participated during manuscript writing. BG and AG edited the final manuscript and approval the version to be published. All authors read and approved the final manuscript.

Abbreviations: ACAPS: Assessment Capacities Project, Crystal VC: Crystal *Vibrio Cholerae*, LPS: Lipopolysaccharide, RDTs: Rapid Diagnostic Methods, TCBS: Thiosulphate citrate bile salt sucrose, WHO: World Health Organization

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