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Yap Boum II, Karl Njuwa Fai, Birgit Schramm, Tchoula Mamiafo Corine, Dora Tchiasso, René Essomba, Donald Buri, Rodrigue Ntone, Lucrèce Eteki, Patricia Mendjime, Mark Ndifon, Nsaibirni Robert Fondze, Eric Youm, Justin Eyong, Ananda L. Kimm-Drapeau, Linda Esso, Georges Alain Etoundi Mballa, Guinevere Q. Lee, Marie-Claire Okomo & Klaudia Porten

Decentralised rapid diagnostic tests enable cholera diagnosis by non-laboratory health workers during outbreaks in Cameroon

Yap Boum II[1,2*], Karl Njuwa Fai[2*], Birgit Schramm[3], Tchoula Mamiafo Corine[3], Dora Tchiasso[3], René Essomba[4], Donald Buri[3], Rodrigue Ntone[3], Lucrèce Eteki[3], Patricia Mendjime[5], Mark Ndifon[3], Nsaibirni Robert Fondze Jr[3], Eric Youm[2], Justin Eyong[3], Ananda L. Kimm-Drapeau[6], Linda Esso[1,5], Georges Alain Etoundi Mballa[5], Guinevere Q. Lee[6], Marie-Claire Okomo[4], Klaudia Porten[3],
1 Faculté de Médecine et de Sciences Biomédicales Université de Yaoundé I, Cameroon
2 Africa CDC, Addis Ababa, Ethiopia
3 Epicentre, Paris, France
4 National Public Health Laboratory, Yaoundé, Cameroon
5 Directorate for Fight Against Diseases, Epidemics and Pandemics, , Yaoundé, Cameroon.
6 Weill Cornell Medical College, New York, USA
Corresponding authors: Njuwa Karl Fai NjuwaF@africacdc.org OR Yap BOUM II BoumY@africacdc.org
* The two authors contributed equally to this manuscript

Abstract

Cholera has re-emerged as a major global public health emergency, with reported cases and deaths tripling between 2022 and 2025 especially in Africa, due to climate shocks, population displacement, fragile water and sanitation systems, and delays in laboratory confirmation. Early detection is critical to interrupt transmission and reduce mortality, underscoring the need for decentralised point-of-care diagnostics.

We conducted a prospective field evaluation of three cholera rapid diagnostic tests (RDTs): SD Bioline Cholera Ag O1/O139, Crystal VC O1, and Cholkit, during active cholera outbreaks in Cameroon. Tests were performed on fresh stool samples by laboratory technicians and by non-laboratory health workers at primary health-care facilities. Diagnostic performance was assessed using PCR as the reference standard. In a subset of samples, RDTs were also performed after alkaline peptone water (APW) enrichment to assess its effect on diagnostic accuracy. Inter-operator agreement and ease of use were evaluated.

Among 492 suspected cholera cases enrolled, 377 samples had PCR results available for analysis. When performed at the point of care, RDT sensitivity ranged from 88% to 95%, comparable to laboratory-based testing, while specificity ranged from 72% to 85%. APW enrichment was associated with a consistent reduction in sensitivity across all three RDTs and reduced specificity for SD Bioline. Inter-operator agreement was high (Cohen’s κ 0·81–0·89). More

than 80% of end users reported that RDTs were easy to use under outbreak conditions.

Cholera RDTs demonstrated high sensitivity, strong inter-operator reliability, and operational feasibility when deployed directly on fresh stool samples by non-laboratory staff. Integrating RDTs into decentralised surveillance systems, without APW enrichment, can accelerate outbreak detection and support timely response in high-burden, resource-limited settings.

Keywords

Cholera; Rapid diagnostic tests; Point-of-care diagnostics; Diagnosis

Introduction

Cholera remains an important global health problem. After several years of declining incidence, the world has witnessed a dramatic resurgence of cholera, with reported cases and deaths tripling between 2021 and 2025[1,2]. This resurgence reflects the convergence of multiple structural drivers, including climate variability and flooding, protracted conflicts, population displacement, urbanization without adequate water and sanitation infrastructure, and persistent inequities in access to health services[3]. Africa has borne a disproportionate share of this burden, experiencing recurrent outbreaks with high case-fatality rates and significant social and economic consequences[4].

Despite long-standing global commitments to cholera elimination, surveillance and response systems in many African countries remain constrained by delayed outbreak detection[3]. Laboratory confirmation, which is traditionally reliant on culture and, increasingly, polymerase chain reaction (PCR), remains essential for definitive diagnosis[5]. However, both methods require infrastructure, trained personnel, reliable specimen transport, and turnaround times that are often incompatible with the rapid pace of cholera transmission. In outbreak settings, delays of several days between case presentation and laboratory confirmation can result in missed opportunities for early intervention, allowing transmission to accelerate and mortality to increase[6–8].

The recent resurgence of cholera has prompted a strategic shift in how the disease is approached within emergency preparedness and response frameworks. Cholera is now formally integrated into Incident Management Support Team (IMST) structures in several regional and continental response architectures[3,9]. This integration reflects the growing recognition that cholera remains a persistent epidemic threat, with a marked increase in scale and impact in recent years, as cases and deaths have tripled between 2022 and 2025.. However, the effectiveness of IMST-led response depends fundamentally on the availability of timely, complete, actionable surveillance data, including early confirmation of outbreaks.In this context, innovation in

cholera diagnostics has emerged as a critical priority. Rapid diagnostic tests (RDTs) for cholera offer the potential to decentralize confirmation, enable earlier outbreak alerts, and support faster deployment of response measures such as case management, water, sanitation and hygiene (WASH) interventions, and oral cholera vaccination[8,10]. RDTs can be performed at or near the point of care, require minimal equipment, and can be used by frontline health workers with limited laboratory training. However, concerns regarding variable specificity and limited evidence from real-world African outbreak settings have constrained their widespread adoption for surveillance and response[11.]

Most existing evaluations of cholera RDTs have been conducted under controlled laboratory conditions or in limited field settings, often focusing on a single product[12]. Data comparing performance across different operator cadres and assessing operational feasibility during active outbreaks remain scarce. Furthermore, the role of RDTs within modern, IMST-driven epidemic management frameworks has not been adequately explored.

This study addresses these gaps by evaluating the diagnostic performance, inter-operator reliability, and usability of three commercially available cholera RDTs during outbreaks in Cameroon. Beyond assessing test accuracy, we situate our findings within the current global cholera resurgence and examine their implications for innovative, decentralized, efficient cholera surveillance and response strategies in Africa and globally.

Methods

Study design and setting

We conducted a prospective field evaluation of cholera rapid diagnostic tests (RDTs) during laboratory-confirmed cholera outbreaks in Cameroon between February 2019 and October 2020. The study was implemented in multiple cholera treatment centres supported by the Ministry of Health and humanitarian partners across five regions: Littoral, South-West, South, North, and Far North. These regions were selected because they experienced active cholera transmission during the study period and represented diverse epidemiological, geographic, and operational contexts, including urban, peri-urban, and remote settings.

Study population

Eligible participants were individuals of all ages presenting to participating treatment centres with acute watery diarrhoea consistent with the World Health Organization (WHO) case definition for suspected cholera in an epidemic context. Written informed consent was obtained from adult participants or from parents or legal guardians of minors; assent was obtained

from older children when appropriate, after obtaining consent from legal guardian or parent. Participants were excluded if they had received systemic antibiotics within the 48 hours preceding presentation or if a stool sample could not be obtained during the observation period.

Rapid diagnostic tests evaluated

Three commercially available cholera RDTs were evaluated: (1) SD Bioline Cholera Ag O1/O139 (Abbott Diagnostics, Yongin-si, Republic of Korea)[13]; (2) Crystal VC O1 (Arkray Healthcare Pvt. Ltd., Surat, India)[14]; and (3) Cholkit (New Horizons Diagnostics, Kolkata, India)[15]. These tests were selected based on prior technical review, availability in the study setting, and relevance for outbreak detection in resource-limited contexts. All assays are designed for qualitative detection of *Vibrio cholerae* serogroup O1, with SD Bioline Cholera Ag O1/O139 also detecting serogroup O139.

Specimen collection and testing procedures

Fresh stool samples were collected from each participant at presentation. At the point of care, nurses performed RDTs according to manufacturers' instructions without additional study-specific training, to reflect routine outbreak conditions. In parallel, trained laboratory technicians independently performed the same RDTs on aliquots of the same specimens in nearby laboratory facilities. Additional aliquots were prepared for molecular testing and transported to a reference laboratory under standard conditions. For a subset of samples, laboratory technicians also performed RDTs after incubation in alkaline peptone water to assess the effect of enrichment on diagnostic performance.

Reference standard

PCR is the reference test for the detection of *Vibrio cholerae* O1 (or O139) and was performed at the Institut Pasteur (IP) in Paris, France. All samples were tested for the presence of *V. cholera* with an initial PCR targeting the spacer region between *V. cholerae* species-specific genes. All *Vibrio cholerae* positive samples were also subjected to rfb gene amplification to identify O1 and O139 serogroups. Negative samples were tested for the presence of 16S RNA to identify samples that may be problematic due to missing DNA or the presence of inhibitors. Laboratory personnel performing PCR were blinded to RDT results.

Data collection and analysis

Demographic and clinical data were collected using standardized case report forms. RDT results were recorded separately by nurses and laboratory technicians to ensure blinding between operators. Ease of use and readability

were assessed using structured questionnaires completed by nurses after test performance. Diagnostic accuracy measures, including sensitivity, specificity, positive and negative predictive values, and likelihood ratios, were calculated using PCR as the reference standard. Inter-operator or inter-reader agreement was assessed using Cohen's kappa coefficient, with κ ≥0·80 indicating good agreement. Inter-reader agreement is considered in this paper as the concordance between the laboratory technicians and nurses independently conducting and interpreting the RDT results.

Ethical considerations

The study protocol was approved by national ethics committee of Cameroon (CNERSH ID: 2019/09/1190/CE/CNERSH/SP) and institutional ethic committee of MSF (ID 1779). Written informed consent was obtained from all participants. All study data were securely stored in the protected databases of Epicentre. Data analysis was performed using anonymized datasets only. All procedures were conducted in accordance with ethical principles governing research involving human participants.

Results

Study population

A total of 484 individuals meeting the suspected cholera case definition were enrolled during the study period, and participants with valid RDT tests were included in the inter-operator and inter-reader RDT performance study. Of these, 377 participants had valid PCR results available and were included in the primary diagnostic accuracy analysis PCR testing could not be performed for the remaining samples because of inadequate sample quality at arrival in the laboratory. Among the analysed samples, approximately 40% were PCR positive for *Vibrio cholerae*. Participants covered a wide age range, including children and older adults, with a median age of 19.9 years (IQR: 8 – 36 years). Female participants accounted for 45% of the study population. At presentation, most patients had moderate or severe dehydration. Baseline demographic and clinical characteristics are presented in Table 1.

Table 1. Demographic and clinical characteristic of individuals presenting to cholera treatment centres during outbreaks in Cameroon, 2019 – 2020 (n = 484)

Characteristic	n / N (%)
Total participants	**484**[1]
Age (years)	
Median age (IQR)	19·9 (8 – 36)
Sex	

Characteristic	n / N (%)
Female	218 / 484 (45·0)
Male	266 / 484 (55·0)
Vaccination status	
Not vaccinated	471 / 484 (97·3)
Vaccinated	13 / 484 (2·7)
Antibiotic use prior to presentation	
No	441 / 484 (91·1)
Yes	43 / 484 (8·9)
Clinical presentation	
Diarrhoea	484 / 484 (100)
Vomiting	
No	159 / 469 (33·9)
Yes	310 / 469 (66·1)
Dehydration status	
None	133 / 477 (27·9)
Moderate	265 / 477 (55·6)
Severe	79 / 477 (16·6)
Outcome at discharge	
Recovered	321 / 395 (81·3)
Transferred	51 / 395 (12·9)
Left against medical advice	18 / 395 (4·6)
Deceased	5 / 395 (1·3)

When performed by laboratory technicians, the sensitivity of the three cholera RDTs ranged from 89% to 91%: 89% (95% CI 81·0–95·0) for SD Bioline Cholera Ag O1/O139, 91% (84·0–96·0) for Crystal VC O1, and 90% (79·0–96·0) for Cholkit (Table 2). Specificity ranged from 75% to 85%, with values of 81% (74·0–87·0) for SD Bioline, 75% (67·0–82·0) for Crystal VC, and 85% (76·0–91·0) for Cholkit.

When RDTs were performed by nurses at the point of care, sensitivity remained high and comparable to laboratory-based testing, ranging from 88% to 95%: 88% (80·0–94·0) for SD Bioline, 90% (82·0–95·0) for Crystal VC, and 95% (85·0–99·0) for Cholkit. Specificity among nurse-performed tests ranged from 72% to 83%, with estimates of 81% (74·0–87·0) for SD Bioline, 72% (64·0–80·0) for Crystal VC, and 83% (75·0–90·0) for Cholkit.

Confidence intervals for both sensitivity and specificity overlapped between laboratory technicians and nurses across all three RDTs, indicating no

statistically significant differences in diagnostic performance by operator cadre.

Inter-operator agreement

Diagnostic performance did not differ significantly by operator cadre. For all test modalities, laboratory technician– and nurse-performed RDTs showed overlapping 95% confidence intervals for sensitivity and specificity, with no statistically significant differences observed (Table 2). These findings were corroborated by inter-operator agreement analysis using the Cohen's kappa coefficient. Applying standard interpretation thresholds (κ=0·80 indicating good agreement and κ≥0·90 indicating very good agreement), substantial agreement was observed between laboratory technicians and nurses for SD Bioline (κ=0·84) and Crystal VC (κ=0·81). Agreement was highest for Cholkit, for which inter-operator concordance approached the threshold for very good agreement (κ=0·89).

Across all three cholera rapid diagnostic tests, diagnostic accuracy was consistently high, with limited variation by operator cadre (Table 2, Figure 1). When performed by laboratory technicians, sensitivity ranged from 89% to 91%, while specificity ranged from 75% to 85%. Positive likelihood ratios exceeded 3·5 for all tests and reached values above 5 for Cholkit, indicating good rule-in capacity, whereas negative likelihood ratios were uniformly low (<0·15), supporting strong rule-out performance.

Performance remained robust when tests were deployed at the point of care. Among nurse-performed tests, sensitivity ranged from 88% to 95% and specificity from 72% to 83%, closely mirroring laboratory-based estimates. Cholkit demonstrated the highest sensitivity when used by nurses (95%, 95% CI 85–99) and the strongest overall diagnostic utility, with a positive likelihood ratio of 5·68 and a negative likelihood ratio of 0·06. Predictive values were high across all tests and operator cadres, with negative predictive values consistently exceeding 90%, reflecting strong capacity to exclude cholera among suspected cases during outbreaks (Table 2).

Effect of alkaline peptone water enrichment

Across all three cholera rapid diagnostic tests, pre-incubation of stool specimens in alkaline peptone water (APW) was associated with a consistent reduction in sensitivity (Table 2). For SD Bioline Cholera Ag O1/O139, sensitivity decreased from 89% (95% CI 81–95) with direct testing to 80% (70–88) following APW enrichment. Similar declines were observed for Crystal VC O1, from 91% (84–96) to 87% (78–93), and for Cholkit, from 90% (79–96) to 78% (65–87).

APW enrichment was also associated with a reduction in specificity for SD Bioline, which decreased from 81% (74–87) to 69% (61–76). In contrast, specificity remained stable for Crystal VC and Cholkit following APW enrichment, with overlapping confidence intervals between direct and enriched testing.

Combining laboratory technicians testing with alkaline peptone water resulted in modest reductions in sensitivity and specificity across tests, although likelihood ratios remained within acceptable ranges for outbreak detection. Importantly, confidence intervals for sensitivity and specificity overlapped across operator cadres for all RDTs, indicating no statistically significant differences in diagnostic performance by cadre (Figure 1).

Table 2. Performance of three rapid diagnostic tests for cholera diagnosis compared to PCR among patients presenting to treatment centres during outbreaks in Cameroon, 2019 – 2020 (n = 484)

Test / Operator	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
SD Bioline Cholera Ag O1/O139						
Laboratory technicians	0·89 (0·81–0·95)	0·81 (0·74–0·87)	0·76 (0·67–0·83)	0·92 (0·86–0·96)	4·77 (3·37–6·75)	0·13 (0·07–0·23)
Laboratory technicians + APW	0·80 (0·70–0·88)	0·69 (0·61–0·76)	0·61 (0·51–0·70)	0·85 (0·77–0·91)	2·58 (1·98–3·37)	0·29 (0·19–0·45)
Nurses (point of care)	0·88 (0·80–0·94)	0·81 (0·74–0·87)	0·74 (0·65–0·82)	0·92 (0·86–0·96)	4·59 (3·28–6·42)	0·15 (0·08–0·26)
Crystal VC O1						
Laboratory technicians	0·91 (0·84–0·96)	0·75 (0·67–0·82)	0·71 (0·62–0·79)	0·93 (0·87–0·97)	3·69 (2·75–4·94)	0·11 (0·06–0·22)
Laboratory technicians + APW	0·87 (0·78–0·93)	0·77 (0·69–0·84)	0·71 (0·61–0·79)	0·90 (0·83–0·95)	3·79 (2·77–5·20)	0·17 (0·10–0·29)
Nurses (point of care)	0·90 (0·82–0·95)	0·72 (0·64–0·80)	0·67 (0·58–0·75)	0·92 (0·86–0·96)	3·26 (2·48–4·28)	0·14 (0·07–0·26)
Cholkit						

Test / Operator	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
Laboratory technicians	0·90 (0·79–0·96)	0·85 (0·76–0·91)	0·79 (0·68–0·88)	0·93 (0·85–0·97)	5·85 (3·59–9·54)	0·12 (0·06–0·25)
Laboratory technicians + APW	0·78 (0·65–0·87)	0·85 (0·77–0·92)	0·76 (0·63–0·86)	0·86 (0·78–0·92)	5·26 (3·18–8·71)	0·26 (0·16–0·43)
Nurses (point of care)	0·95 (0·85–0·99)	0·83 (0·75–0·90)	0·76 (0·64–0·85)	0·97 (0·90–0·99)	5·68 (3·66–8·80)	0·06 (0·02–0·19)

- PCR was used as the reference standard.
- APW = alkaline peptone water
- PPV and NPV depend on disease prevalence in the study population.
- Likelihood ratios >5 and <0·2 indicate strong rule-in and rule-out diagnostic utility, respectively.

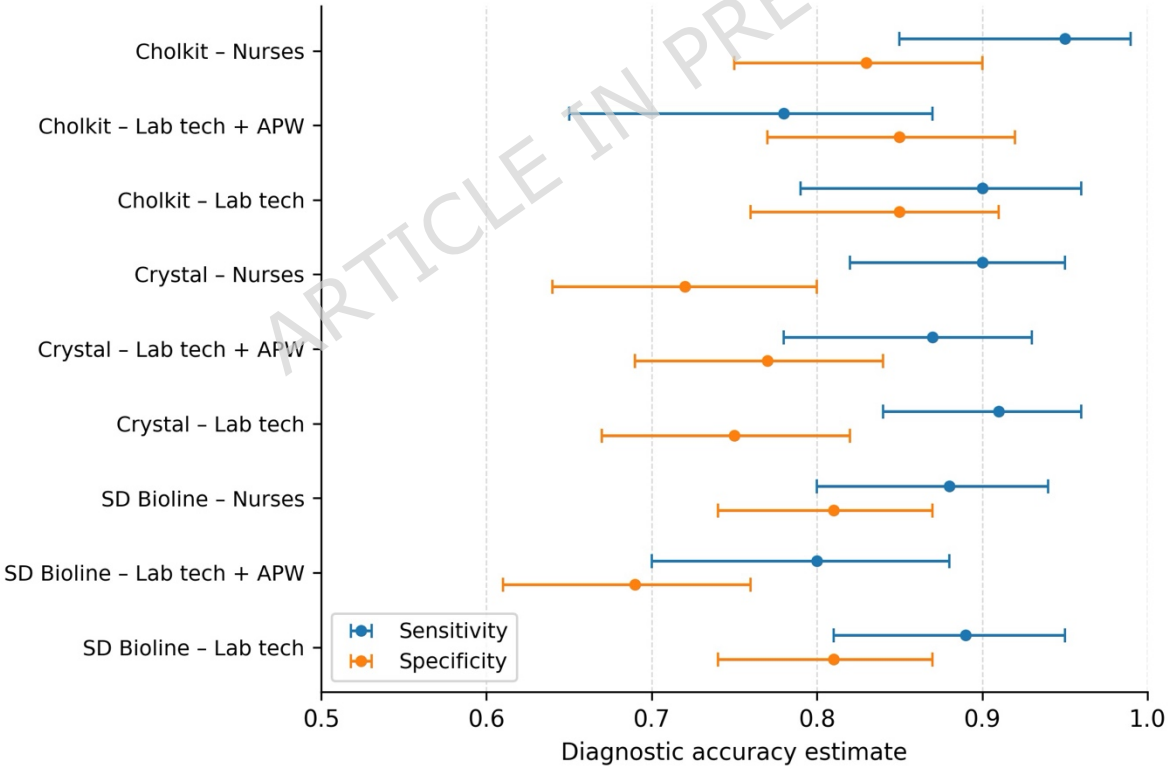

Figure 1. Diagnostic accuracy (sensitivity and specificity) with 95% Confidence intervals of three cholera rapid diagnostic tests stratified by device and health worker cadre during outbreaks in Cameroon, 2019 – 2020 (n = 484)

Ease of use and operational readability of cholera rapid diagnostic tests

Overall usability of the three cholera rapid diagnostic tests (RDTs) was high, although important differences in operational performance were observed (Table 3). SD Bioline Cholera Ag O1/O139 and Crystal VC O1 were reported as easy to use by 92·3% and 90·6% of end users, respectively, whereas Cholkit was rated as easy to use by a lower proportion of users (81·1%). Difficulties in result read-out were reported in 7·7% of SD Bioline tests, 9·4% of Crystal VC tests, and 18·9% of Cholkit tests.

Among tests with reported read-out difficulties, the nature of the challenges varied substantially by RDT type (Figure 2). For SD Bioline and Crystal VC, irregular or coloured test backgrounds were the most frequently cited issues, accounting for 58·3% and 51·2% of reported difficulties, respectively. In contrast, Cholkit was predominantly affected by very weak test lines, which represented 82·1% of readability issues, indicating a specific limitation related to visual signal intensity rather than background artefacts. Unclear instructions were rarely reported across all tests (<3%), suggesting that user comprehension was not a major contributor to operational challenges.

Taken together, Table 3 and Figure 2 demonstrate that while all three RDTs are feasible for decentralised deployment, differences in result readability may influence user confidence and interpretation at the point of care.

Table 3 Ease-of-use of three cholera rapid diagnostic tests as reported by nurses under field conditions at cholera treatment centres during outbreaks in Cameroon, 2019 – 2020 (n = 484)

Indicator	SD Bioline (n=465)	Crystal VC (n=438)	Cholkit (n=297)
Easy to use	429 (92·3%)	397 (90·6%)	241 (81·1%)
Difficulties in result read-out	36 (7·7%)	41 (9·4%)	56 (18·9%)
Reasons for difficulties*			
Irregular/coloured background	21 (58·3%)	21 (51·2%)	6 (10·7%)
Very weak test lines	11 (30·6%)	17 (41·5%)	46 (82·1%)
Instructions not clear	1 (2·8%)	1 (2·4%)	1 (1·8%)
Missing responses	3 (8·3%)	2 (4·9%)	3 (5·4%)

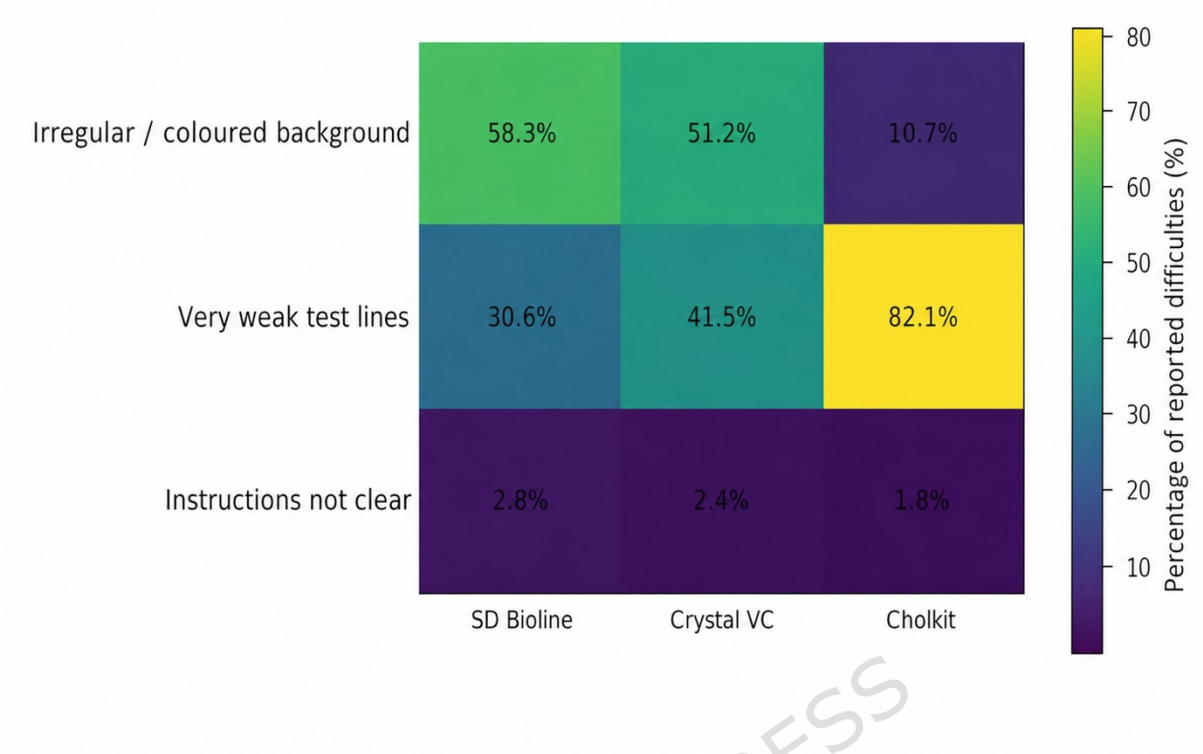

Figure 2. Heat map of reasons for result read-out difficulties across three cholera rapid diagnostic tests as reported by nurses under field conditions during outbreaks in Cameroon, 2019 – 2020 (n = 484)

Discussion

The tripling of global cholera cases and deaths between 2022 and 2025 highlights the limitations of traditional surveillance models that rely heavily on centralized laboratory confirmation. Our findings demonstrate that cholera RDTs can be effectively deployed at the point of care by frontline health workers, achieving high sensitivity and strong inter-operator reliability during active outbreaks.

These results are particularly relevant in Africa, where delays in laboratory confirmation often impede early response. By enabling faster detection of outbreaks, RDTs can serve as a critical component of epidemic intelligence, allowing health authorities to act before transmission becomes widespread.

Considering the previously stated WHO minimum recommended performance of a Cholera RDT as having a sensitivity of at least 90% and specificity of at least 85%[16], Cholkit (as operated by lab techs and by paramedical staff) met the desired minimal performance. SD Bioline and Crystal reached the sensitivity target but fell short a with lower than desired 85% specificity. Our findings are closely in line with those of a recently conducted meta-analysis

which combined the findings of 20 available studies (11 in Africa) on test performance of overall eight different Cholera RDTs (including SD Biosensor and Crystal)[17], reporting an overall performance estimate of 91% sensitivity and 80% specificity. Notably, the meta-analysis also indicated that RDT sensitivity was higher in studies conducted in Africa (92%) compared to Asia (82%), while specificity was lower in studies conducted in Africa (83%) than in Asia (90%)[17].

The specific Target Product Profile (TPP), which was developed by the Global Task Force on Cholera Control in collaboration with cholera screening experts and RDT users with a main objective of supporting research and development of products that address unmet priority needs, specified the minimum characteristics of an RDT that would be useful in the field as a clinical sensitivity of at least 90% and a specificity of 95%[18]. The present evaluation of the three RDTs under laboratory-conditions or at the point-of-care by nurse, indicated that all three were close to the desired sensitivity, while none of the three candidate tests met the more stringent requirements of 95% specificity. As discussed recently by Azman et al., based on currently available RDTs and their performances, it is unclear whether the specificity of any RDTs used directly on stool can meet this target product profile[19]. Positive likelihood ratios supported the use of RDTs for outbreak detection, while negative likelihood ratios were sufficiently low to provide reassurance in ruling out cholera among suspected cases in epidemic contexts.

A key strength of this study is the demonstration of consistent performance across operator cadres. The high inter-operator agreement observed confirms that cholera RDTs can be reliably used by frontline health workers without compromising diagnostic accuracy. Our nurses operated the RDTs without any formal pre-study training which may be attributed to the fact that in our setting, many had substantial prior experience with similar outbreaks and were routinely involved in the use of other POC RDTs like malaria in their daily clinical practice. This finding has major implications for outbreak response, where laboratory personnel may be scarce, overwhelmed, or geographically distant from points of care. This corroborates a similar evaluation study using three different assays in Bangladesh and confirms that well-trained paramedical staff like nurses can replace laboratory technicians in remote areas[20.] It also projects the possibility of task-shifting from the laboratory to point-of-care by using paramedical staff for cholera testing including community healthcare workers in emergency response situations[20]. Task-shifting of diagnostic enable decentralization of detection and faster feedback loops between communities and response teams.

The study also assessed whether an additional step during specimen preparation (i.e., enrichment in APW for 4 to 6 hours) would potentially improve the performance of the tests. Overall, no performance gains in the three RDTs were observed by use of this additional step. Indeed, enrichment

in APW may be more relevant when supporting the detection of *V. cholerae* in specimens where few organisms are present (as for convalescent patients or asymptomatic carriers), while unnecessary for specimens collected from patients in early stages of the disease passing liquid stool[21]. In outbreak contexts, diagnostic strategies should prioritize speed, simplicity, and scalability. Additional laboratory steps introduce delays, increase biosafety risks, and may reduce sensitivity. These findings argue against routine enrichment prior to RDT use during outbreaks.

In addition, diagnostic performance of RDTs is influenced by the timing of testing relative to the course of infection, as pathogen load varies over time and may affect test sensitivity[22.] Our analysis was limited to symptomatic cases and did not include data on time since symptom onst, precluding assessment of how sensitivity changes across different stages of illness. Furthermore, asymptomatic infections may contribute to transmission and could be associated with lower pathogen loads and reduced detectability.

RDTs should not be viewed as replacements for laboratory confirmation but as complementary tools designed to bridge critical time gaps during outbreaks. When integrated into surveillance systems and linked to confirmatory testing, RDTs can strengthen early warning systems and enhance epidemic preparedness.

This study was conducted during active cholera outbreaks, and the findings may not be directly generalisable to low-prevalence or inter-epidemic settings. Diagnostic performance, particularly positive predictive value, is influenced by disease prevalence, and therefore the positive predictive values observed in this study may be lower in settings with sporadic transmission or during routine surveillance periods[23.] In addition, the moderate specificity observed for some RDTs limits their use as stand-alone tools for individual case confirmation.

However, these limitations are intrinsic to the intended use of cholera RDTs and do not detract from their public health utility. The primary purpose of RDTs is to support early outbreak detection, rapid situational assessment, and timely initiation of control measures, rather than definitive individual diagnosis. When integrated into decentralised surveillance systems and complemented by confirmatory laboratory testing, RDTs provide a valuable, rapid screening tool that can strengthen outbreak preparedness and response, particularly in resource-limited and high-risk settings.

A notable limitation observed in this study is the high proportion (>80%) of very weak test lines reported with the Cholkit RDT. This finding raises important concerns regarding the interpretability and reliability of results in field settings, particularly when used by non-laboratory health workers. Very weak lines may increase inter-reader variability and contribute to reduced

specificity, especially in high-pressure outbreak contexts where rapid decision-making is required. These challenges underscore the critical need for standardized training, clear interpretation guidelines, and quality assurance mechanisms to support consistent use of RDTs. Furthermore, this limitation highlights the importance of continued evaluation and optimization of RDT performance under real-world conditions in African outbreak settings.

Conclusion

In the context of a rapidly increasing global cholera burden, strengthening early detection at the periphery of the health system is critical. This study demonstrates that cholera rapid diagnostic tests can be performed and interpreted by non-laboratory health workers at the point of care, with diagnostic performance approaching that of laboratory-based testing. While direct testing of fresh stool specimens without prior alkaline peptone water enrichment did not fully meet the Target Product Profile criteria, it showed acceptable performance for use in outbreak settings, particularly where rapid decision-making is essential.

In such contexts, prioritizing sensitivity to enable early detection and initiation of control measures may be more critical than achieving optimal specificity, especially while awaiting confirmatory laboratory results. However, it is important to emphasize that a single negative result should not be used to rule out cholera, and appropriate clinical and epidemiological judgment remains essential.

Integrating cholera RDTs into decentralized surveillance systems therefore offers a pragmatic and scalable approach to accelerate outbreak detection, enable earlier response, and reduce transmission and preventable mortality. Expanding access to point-of-care diagnostics at primary health-care and community levels represents a key opportunity to strengthen epidemic preparedness and response in high-burden, resource-limited settings.

Author contribution statement

Y.B.II. and K.P. conceived and designed the study. K.N.F., T.M.C., D.T., R.E., D.B., R.N., L.E., P.M., M.N., N.R., J.E., L.E., and M.-C.O. conducted data collection and field activities. K.N.F., B.S., E.Y., N.R., and M.N., curated and validated the data. K.N.F., B.S., E.Y., M.N. and Y.B.II. performed data analysis and interpretation. Y.B.II., L.E., G.A.E.M., and M.-C.O. supervised the study. K.N.F. and Y.B.II. drafted the manuscript.

Additional Information

Authors declare no competing interests.

Legend

Table 1. Demographic and clinical characteristic of individuals presenting to cholera treatment centres during outbreaks in Cameroon, 2019 - 2020

Table 2. Performance of three rapid diagnostic tests for cholera diagnosis compared to PCR among patients presenting to treatment centres during outbreaks in Cameroon, 2019 – 2020 (n = 484)

Table 3 Ease-of-use of three cholera rapid diagnostic tests as reported by nurses under field conditions at cholera treatment centres during outbreaks in Cameroon, 2019 – 2020 (n = 484)

Figure 1. Diagnostic accuracy (sensitivity and specificity) with 95% Confidence intervals of three cholera rapid diagnostic tests stratified by device and health worker cadre during outbreaks in Cameroon, 2019 – 2020 (n = 484)

Figure 2. Heat map of reasons for result read-out difficulties across three cholera rapid diagnostic tests as reported by nurses under field conditions during outbreaks in Cameroon, 2019 – 2020 (n = 484)

Data availability statement

The datasets generated and/or analyzed during the current study are not publicly available due to ongoing reports that are not yet prepared but are available from the corresponding authors on reasonable request.

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