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Evaluation Of The Diagnostic Performance Of A Rapid Immunochromatographic Test (RDT) Compared To Real-Time PCR (Radione System) In The Context Of The Cholera Epidemic In The Democratic Republic Of Congo

Cesar Christian CARRENARD ODZALI[1,2] , Dieudonné Mwamba KAZADI[1] , Tatiana BUABA[4] , Declerk KAPAY LEMBUSA[2] , Destin MBONGI MOKE[1,3] , Boniface KABASELE[6] , Fiston KADIATA[6], Patricia MBUNZU NDIBAYOLA[8] , EPENDA LOKAKAO Ruphin[7], Odette KABENA NGANDU[2] , Erick KAMANGU NTAMBWE[1,3]

[1]National Institute of Public Health
[2]University of Kinshasa Faculty of Sciences, Life Sciences Department
[3]University of Kinshasa Faculty of Medicine
[4]Kinoise Clinic, Kinshasa
[5]ISTM DIMBELENGA, Kananga
[6]ISTM TSHIMBULU, Kananga
[7]Directorate of Epidemiological Surveillance , Kinshasa
[8]Pistis Medical Center

Corresponding Author : Cesar Christian CARRENARD ODZALI, cesarcarrenard1@gmail.com

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Summary

Introduction : In the Democratic Republic of Congo (DRC), rapid diagnosis of cholera is crucial for epidemic response. This study evaluates the performance of the immunochromatographic rapid diagnostic test (RDT) compared to real-time PCR (RADIOne system).

Materials and Methods : A cross-sectional study was conducted on 264 stool samples initially tested positive by rapid diagnostic tests (RDTs) in the Democratic Republic of Congo (DRC). All samples were analyzed using the RADIOne 5-Plex Diarrhea multiplex panel, capable of simultaneously detecting *Vibrio cholerae* (ctxA gene), *Shigella, Salmonella* , and *E. coli* (ETEC/STEC). Positive predictive values (PPVs) and 95% confidence intervals (CIs) were calculated.

Results : PCR confirmed *Vibrio cholerae* in 210 of the 264 RDT-positive cases, for a positive predictive value (PPV) of 79.55% (95% CI: 74.07%–84.14%). In the 54 unconfirmed cases (20.45%), multiplex PCR revealed high circulation of other enteropathogens : *Shigella spp.* (45.46%) and ETEC-producing *E. coli* (32.58%). Overall, 92.42% of the RDT-positive samples contained at least one target pathogen.

Conclusion : Although the rapid diagnostic test (RDT) is an essential screening tool, it presents a significant risk of false positives due to cross-reactions with *Shigella and E. coli.* The integration of point-of-care PCR is recommended to validate epidemic alerts and optimize differential clinical management in the DRC.

Keywords : Cholera, RDT, PCR RADIOne, Democratic Republic of Congo, Diagnostic performance, Cross-reactions.

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1. Introduction

In May 2011, the World Health Assembly recognized the re-emergence of cholera as a major global public health problem. In recent years, the incidence of cholera has increased steadily: the World Health Organization (WHO) recorded approximately 317,000 cases and 7,500 deaths in 2010, representing a 43% increase in the number of cases and a 52% increase in the number of deaths compared to 2009 (WHO, 2011).

The Democratic Republic of Congo (DRC) is facing a resurgence of the cholera epidemic, which is worsening and affecting 20 of its 26 provinces. Poor sanitation conditions and conflicts are facilitating the spread of *Vibrio cholera,* particularly during the rainy season (WHO, 2023).

Early detection and confirmation of an outbreak are crucial for the rapid implementation of appropriate interventions. While culture is necessary for the confirmation and characterization of the epidemic strain, rapid diagnostic tests (RDTs) are probably the most promising tools for early detection in areas lacking laboratory resources (Page et *al.,* 2012).

Diagnosis is traditionally based on clinical presumption and biological confirmation. According to the WHO, an effective test must be reliable, rapid, reproducible, easy to perform, well-accepted by target populations, have few side effects, and be moderately costly (HAS, 2004). However, the emergence of new methods such as point-of-care PCR necessitates rigorous comparative evaluation (Dick et *al* ., 2012) .

In most of these assessments, stool culture is used as the reference method to estimate performance. Although it remains the reference method for laboratory cholera surveillance, stool culture cannot be considered a foolproof method due to its lack of sensitivity (Branscum et *al* ., 2005).

To address this issue, a combination of techniques can be used to improve the reference standard, most often culture combined with PCR. While PCR can be used on stool samples to detect *V. cholera-specific DNA targets,* and while strains O1 and O139 are not validated as references for cholera diagnosis, their theoretical ability to detect small amounts of bacteria or dead cells suggests they could improve the sensitivity of a new reference method (Limmathurotsakul D et *al.,* 2010).

The emergence of new rapid screening methods necessitates a rigorous evaluation of their performance compared to standard PCR, in order to verify their sensitivity, specificity, limit of detection, and accuracy. This study aims to compare the diagnostic efficacy of the rapid cholera screening test (RDT) to that of real-time PCR (RADIONE Molecular System).

2. MATERIALS AND METHODS

2.1. Sample collection

Rectal swabs were collected from patients meeting the case definition of acute watery diarrhea. The samples were transported in a Carry Blair environment to the laboratory located at the Clinique Kinoise in Kinshasa, Democratic Republic of Congo.

2.2. Analysis by Rapid Diagnostic Test (RDT)

V. cholerae O1 and *O139* antigens via monoclonal antibodies conjugated to colloidal gold.

2.3. PCR analysis (RADIONE system)

The RADIONE 5-Plex *Diarrhea panel* was used as the reference test. This real-time PCR system allows:

- Automated closed-circuit nucleic acid extraction.
- Simultaneous detection of *V. cholerae (* ctxA gene), *Salmonella* , *Shigella, STEC* and *ETEC.*

Achieving results in 60 minutes.

2.4. Data analysis

The information recorded on the data collection sheets was entered into Excel 2016 software. A data entry mask was developed, and the data were entered and analyzed using R software version 4.5.3, such as predictive (positive) values, confidence interval, and others.

Estimation of sensitivity and specificity using a reference standard

The following definitions were used for the analysis using a reference standard:

Rapid Diagnostic Test (RDT) Performance Standards: The RDT is considered a screening test. Its reference standards, according to the WHO and the GTFCC (Global Task Force on Cholera Control), are: - **Expected sensitivity** : > 90%. The RDT must be able to detect almost all true cases to prevent an outbreak from going undetected. - **Expected specificity** : 60% to 80%. Lower specificity (more false positives) is accepted in exchange for a rapid response .

RADIOne PCR Performance Standards: PCR is considered a confirmatory method or "gold standard" alternative when culture is not possible. - **Limit of Detection (LOD**): The standard for a high-quality PCR like RADIOne is to detect approximately 10 $^{\wedge 3}$ copies/ml. - **Analytical Specificity** : Close to 100%. The PCR should not react to bacteria other than those targeted by its specific primers. - **Multiplex Panel** : The standard for diagnosing diarrhea is the inclusion of the main targets: *Vibrio cholerae, Shigella spp ., Salmonella, and E. coli (ETEC/EPEC).* Your study meets this standard by identifying the causes of false positives in the RDT.

Diagnostic validation standards : To evaluate the performance of a diagnostic test such as the RDT against a "Gold Standard" (PCR), the scientific community refers to the STARD (Standards for Reporting Diagnostic Accuracy) standards (Bossuyt et al., 2015; Stard , 2015).

Sensitivity and specificity were measured respectively as the proportion of samples positive on the rapid diagnostic test (RDT) among samples positive on the reference method, and as the proportion of samples negative on the RDT among samples negative on the reference method. Exact 95% binomial confidence intervals were determined.

Inter-batch reproducibility was estimated using Cohen's kappa coefficient

3. RESULTS

3.1. Diagnostic Performance Summary

The study included a total of 264 samples initially tested positive by RDT in the field; PCR analyses revealed the following data illustrated in Figure 1.

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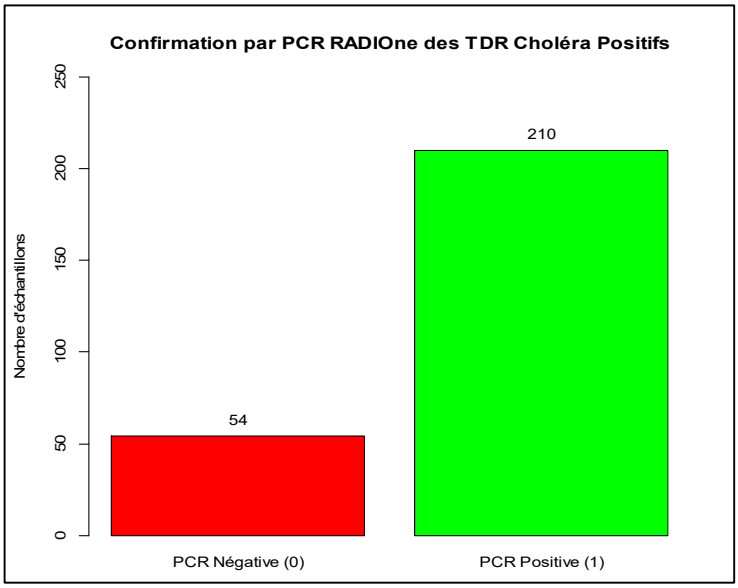

Figure 1: Distribution of RADIOne PCR results for my samples initially positive by the Cholera RDT

The green bar (1) represents true positives (n=210), i.e., samples where the diagnosis of *Vibrio cholerae* was confirmed by PCR. The red bar (0) illustrates cases not confirmed for cholera (n=54), suggesting cross-reactions with other enteropathogens such as *Shigella* or *E. coli* detected by the multiplex panel. The calculated Positive Predictive Value (PPV) is **79.55%** within the 95% confidence interval (CI) of **74.07%–84.14%.**

3.2. Analysis of Cross-Reactions and Co-infections

The main value of this analysis lies in the identification of pathogens present in the 54 cases where PCR was not confirmed for cholera. Multiplex PCR revealed a high circulation of other enteropathogens (Figure 2).

The total exceeds 100% because several samples showed co-infections, therefore several pathogens were detected for the same sample.

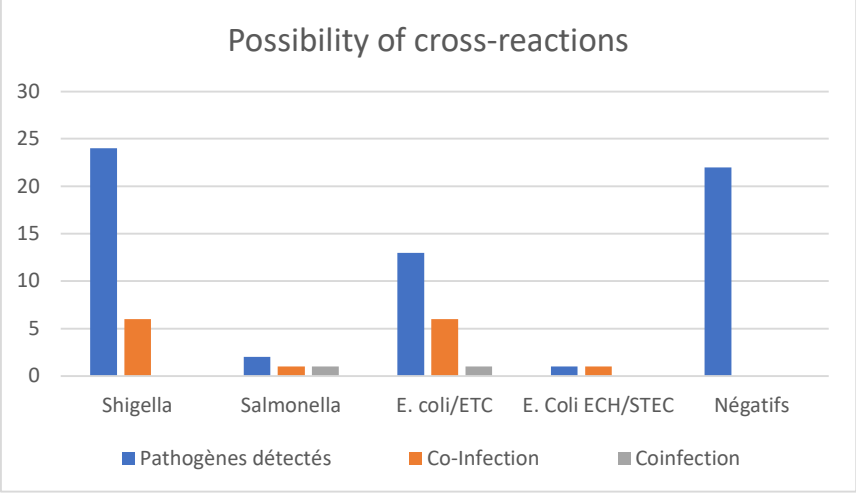

Figure 2: Identification of other enteropathogens (Multiplex PCR)

Figure 2 highlights that *Shigella spp.* is the main cross-reactive agent (present in nearly half of all cases), which justifies our recommendation to use multiplex tests during epidemics. This also explains the variations in observed specificity.

3.3. Distribution of bacterial loads (CT value)

Analysis of the RADIOne PCR threshold cycles shows a high bacterial concentration for confirmed cases, which strengthens the reliability of the confirmation:

- **Average CT for Cholera** : Approximately **28.4** (indicating a high bacterial load validating the quality of the extraction and amplification of the RADIOne system).
- **Range** : Values vary from **16.52** (very high load) to 38.10 (detection limit).

3.4. Cohen's Kappa Coefficient

This coefficient allows us to measure whether the concordance between our RDT and the RADIOne PCR is due to chance or to real diagnostic performance (Figure 3)

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Cohen's Kappa for 2 Raters (Weights: unweighted)

Subjects = 264
  Raters = 2
   Kappa = 0
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Figure 3Cohen's Kappa coefficient results

Since the TDR always gives the same result (100% positive in our sample of 264), the agreement "expected by chance" becomes equal to the observed agreement, which mathematically cancels out the result.

3.5. Spatial Distribution of Cases

The data shows that the majority of samples come from the province of Kinshasa, with a particular concentration in certain health zones:

- **Limete area : predominant area in our study (** Kiganbwa and Kawele areas) concentrates the largest volume of samples at the beginning of the study.
- **Kalamu II area** : Heavily represented in the latest records (Yolo area) in the more recent phase of the response.

These results suggest that the positive rapid diagnostic test (RDT) could be linked to immunological cross-reactions with other enteropathogens circulating in Kinshasa's health zones, thus reflecting the capital's urban ecology. This spatial distribution also underscores the endemicity of cholera in densely populated urban areas with inadequate sanitation.

4. Discussions

Molecular methods such as PCR have proven to be a sensitive and specific technique compared to others capable of performing microscopic examination for the diagnosis of parasitic stools. However, these analyses can be challenging. They are limited by a number of factors, such as the difficulty in isolating the genetic material (DNA) of these protozoa, which are present as oocysts or cysts with very robust cell walls that hinder disruption of the organisms and the absorption of lysine (Surl et al., 2011).

The presence of certain fecal constituents such as hemoglobin degrades enzyme products , bilirubin, bile salts, and calcium from carbohydrates, which can alter and/or inhibit the metabolism of these products. PCR amplification can lead to false negatives and false positive PCR results (Oikarinen et al., 2009).

On the other hand, the requirement for a separate reaction for each parasite and pre-analytical complex, the handling of samples for PCR tests could make the analyses quite time-consuming, in addition to their high costs (Schrader et *al* ., 2012).

The use of Point-of-Care (POC) PCR represents a major technological leap for the DRC. Unlike conventional culture, which requires 48 to 72 hours, the RADIOne system enabled confirmation in less than 90 minutes.

This speed is in line with the recommendations of the GTFCC (2022), which advocates the integration of decentralized molecular biology to avoid false epidemic alerts and optimize the use of oral vaccine stocks.

The Positive Predictive Value (PPV) of 79.55% for cholera should be interpreted with caution. The high presence of *Shigella* (45.46%) and E. *coli* (32.58%) in these same samples suggests that the RDT may exhibit cross-reactivity or that patients in the DRC suffer from multiple enteric pathologies (Naglaa F et *al* ., 2022) . However, the lower bound of the confidence interval at 74.2% indicates that accuracy could decrease under less controlled field conditions. This result is slightly higher than that reported by Moser et *al* . (2024) in Goma, who observed a PPV fluctuating between 65% and 75% depending on the phase of the epidemic. This difference can be explained by the high prevalence of Vibrio cholerae in Kinshasa during our study period, as the PPV is directly related to the prevalence of the disease in the tested population.

The study noted that 92.42% of positive RDTs were confirmed by at least one PCR target. This indicates that the RDT is a good initial screening tool, but its reliability in specifically identifying Vibrio cholerae remains to be confirmed by a molecular method.

Regarding the performance of rapid diagnostic tests (RDTs) in the Democratic Republic of Congo, Moser et *al* . (2024) showed that the sensitivity and specificity of RDTs such as Crystal VC vary considerably depending on the context. This leads us to conclude that in high-incidence areas, RDTs remain an essential screening tool, but that biological confirmation is crucial to avoid overestimating the extent of an epidemic.

The RDT can "confuse" the surface proteins or toxins of *Shigella* and E. *coli* with those of *V. cholerae ,* which technically justifies the percentages of unconfirmed results. These findings are supported by publications from the CDC (Review Report, 2023) and studies such as that by Bonten et *al. (2021), which found that* the surface antigens of certain enterotoxigenic (ETEC) Shigella and E. coli strains share *structural* similarities with the lipopolysaccharides of *V. cholerae O1* , potentially saturating the RDT's capture antibodies and leading to false positives.

5. Conclusion

This comparative study highlights the strengths and limitations of rapid diagnostic tools in the cholera response in the Democratic Republic of Congo. While the Rapid Diagnostic Test (RDT) remains an essential first-line tool for early detection, our analysis reveals a Positive Predictive Value (PPV) of 79.55%.

The use of the RADIOne PCR system made it possible to identify that nearly 20% of cases reported as cholera by RDT were actually false positives, mainly due to cross-reactions with other enteric pathogens such as *Shigella* (45.46%) and *E. coli* (32.58%).

Although the RDT is a valuable rapid tool in the context of an epidemic, it presents a significant risk of false positives in areas where other enteric bacteria are circulating simultaneously.

We recommend a revision of the surveillance algorithm by health authorities and decision-makers: A cholera outbreak in a new health zone should no longer be declared based solely on rapid diagnostic tests (RDTs). Systematic confirmation by multiplex PCR (such as RADIOne) should be required to validate the first case.

Decentralization of Point-of-Care PCR: Investing in closed-circuit PCR (POC) technologies in "hotspot" areas to reduce the time required to return results, which is currently too long with conventional culture.

Differential management: In the presence of a patient with a positive RDT but a poor response to standard cholera treatment, rapidly consider Shigella or other enterobacteria infection, in accordance with the co-infection rates observed in this study.

Stock management: Use PCR confirmation data to adjust orders of oral rehydration salts (ORS) and vaccines, to avoid wasting resources in non-choleric diarrhea outbreaks.

Specificity Studies: Conduct further research including negative RDT samples to calculate the overall sensitivity and specificity of the test in the Congolese context.

Cross-Reactivity Analysis: Investigating the molecular causes of cross-reactions between RDT antibodies and circulating Shigella antigens in the DRC to improve the accuracy of future diagnostic kits.

Sensitivity , ranging from 58 to 100%; and specificity, which would vary between 60 and 100% depending on the test and the context, even taking into account the limitations of the study methodology which could have led to an underestimation of specificity 7,8

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