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Assessment of the SD Bioline Rota/Adeno Antigen-Based Test in Infants with Diarrhea by RT-PCR

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KEYWORDS
Diarrheal and non-diarrhea patient , RDT vs. RT-qPCR, SD Bioline Rota/Adeno antigen test, children.

ABSTRACT:
Introduction: Intestinal imbalances cause diarrhoea. Three or more daily loose stools, severe if under 14 days. Chronic diarrhoea has non-infectious causes and severe infections. Rotaviruses, especially RVA genotype G/P, are essential. Affordable RDTs have varying accuracy, but ELISA and RT-PCR are diagnostic procedures. Leukocyte, stool culture, and pH tests pinpoint reasons. C. difficile and E. coli must be tested. Parasite testing improves diagnosis.
Aims and Objective: This study uses RT-PCR and Ct value to evaluate the newly designed antigen test based Rapid Diagnostic Test (RDT) kit for identification of new-born diarrhoea rotavirus A.
Methods: A cross-sectional study from September 2022 to August 2023 used RT-PCR to detect RVA in faeces. Asymptomatic children under five and diarrheal children were sampled. After extracting viral RNA with the RDT, RT-qPCR targeting NSP4 confirmed RVA presence. VP7 and VP4 gene genotyping was performed on samples with Ct < 39, using a CT cut-off of 24 for gastroenteritis assessment.
Results: Table 1 shows baseline characteristics of diarrheal and non-diarrhea patient counts with diarrhoea, high Ct values, and symptoms are shown in Figure 1. RT-qPCR and RDT virus strain identification findings are shown in Table 2. RDT vs. RT-qPCR diagnostics are shown in Figure 2. Table 3 and Table 4 examine RDT's diarrhoea detection and Ct value detection abilities. RT-qPCR and RDT results agree in Figure 3, showing RDT's specificity but lower sensitivity.
Conclusion: The study has concluded that the accuracy of designed RDT is considerable in the presence of diarrhea and can be used by common people in ruling out Rotavirus a infection, but cannot be the ultimate diagnostic tool.

1. INTRODUCTION

For babies and early children, the usual about 10 mL/kg/day of water is found in faeces; for teens and adults, it is 200 g/day. Due to an imbalance in the healthy operation of the large and small intestine's physiological processes that are liable for the ingestion of different ions, other substrates, including ultimately water, diarrhoea is the increase in the amount of water in stools [1]. Three or more stools that are watery or loose per day over a period of serious diarrhea, is defined as lasting 14 days or less. Diarrhoea is regarded as chronic and persistent whenever an episode lasts over fourteen days. Severe diarrhoea is usually brought on by infection. The prevalence of noninfectious aetiologies increases when diarrhoea becomes persistent [2]. This distinction is important as the length and unique aetiology of the ailment influence how to manage and cure it. All individuals with diarrhoea need rehydration treatment as part of their care. Good hand washing is a crucial part of preventing contagious diarrhoea because it prevents the sickness from spreading [3].

While "acute diarrhoea" is a more accurate description of the latter, "acute gastroenteritis" is often used interchangeably. The word gastroenteritis denotes both stomach and small intestine involvement, although even though acute diarrhoea

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is the infectious form of diarrhoea, realistically speaking, gastric involvement was almost never found in acute diarrhoea. Additionally, enteritis is not always present. Instances of infectious diarrhoea absent enteritis include cholera and shigellosis. Consequently, it is more clinical to refer to the condition as acute diarrhoea instead of acute gastroenteritis appropriate [4].

Over 500,000 fatalities globally, mostly in poor nations, are attributed to diarrheal infections every year, making them one of the main factors contributing to morbidity and death in young children under five. One of the most common rotaviruses is group A (RVA), which is a member of the Reoviridae family viruses responsible for gastroenteritis in children [5]. The triple-layered, non-enveloped viral particle's genome is made up of eleven double-stranded segments of RNA (dsRNA) code encode NSP1 to NSP6 are six non-structural and six structural proteins (VP1 through VP4, VP6, and VP7). The RVA G/P genotypes are determined by two of these segments' outer capsid proteins, VP7 (glycoprotein or G) & VP4 (spike protease-sensitive and P] [6]. Characterization of the viruses utilising the full genome sequencing covering all eleven parts is beneficial in addition to establishing the important role of reassortants into the introduction of new RVA variants into the human population G/P kinds of rotaviruses. Globally, the strains that infect people the most often and widely is G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] [7].

There were an estimated 128,500 paediatric deaths from diseases connected with RVA in 2016. Therefore, an early and precise RVA diagnosis is crucial for the therapy of particular patients, for post-treatment care, and for population-based testing programmes for efficient prevention. There are several diagnostic methods available to find rotaviruses. RVA virus particles were originally discovered using electron microscopy [8]. However, because of its high cost, knowledge need, and lack of sensitivity, this technology is not frequently used. When compared to results from electron microscopic analysis, enzyme-linked immunosorbent tests (ELISA), which have been frequently employed since the 1980s, produce good results [9]. With the benefit of greater sensitivity and specificity, recent diagnostic procedures have been superseded by molecular methods like PCR. On-site disease testing is difficult in underdeveloped nations due to the high cost of the equipment and sophisticated technical requirements for these methods [10]. Additionally, rotavirus infections are detected by RT-PCR up to 14% more frequently than by ELISA for a group of healthy controls, indicating that rotavirus a might not always be the source of infectious diseases that are RT-PCR positive. In this regard, a number of straightforward and rapid immunochromatographic diagnostic tests (RDTs) at reasonable prices have been made available for purchase [11]. Such RDTs don't need complicated laboratory equipment or in-depth reader training. They therefore appear to be especially helpful in healthcare facilities with limited resources such tests might enhance patient care and community-based screening programmes in low- and middle-income nations. Their effectiveness is still debatable, and the outcomes achieved in asymptomatic people may still be in doubt due to low virus levels [12].

Acute diarrhoea patients often suffer from a self-limited course & don't need lab work or imaging. In order to rule out infections in a patient having severe sickness or bloody diarrhoea, a stool culture was necessary. Shiga toxin & lactoferrin tests must be done in addition if bloody stools are present. Testing in Clostridium difficile infection is necessary for patients who have recently used antibiotics or have been hospitalised. In a patient suffering severe diarrhoea, imaging is not typically prescribed. However, if a patient exhibits strong peritoneal symptoms, an abdomen CT may be necessary [13].

The presence of too many reducing chemicals or a stool pH under 5.5 is signs of carbohydrate intolerance in diarrhoea, which is typically a complication of viral infections. It is ephemeral in character. Neutrophils along with other leucocytes are excreted into the stool as a result of enteroinvasive infections that damage the large bowel. Leukocytes in the stools rule out the presence of viruses, Vibrio, and enterotoxigenic E coli [14].

The stool sample should be kept chilled at 4°C and placed into a transport medium if it cannot be cultured after two hours after specimen collection. Stool cultures have a poor yield, but when they are healthy, they can be useful. If there are symptoms of

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colitis or if there is faecal leucocytosis, Shigella, Campylobacter, Yersinia enterocolitica, and C Difficile should all be cultured from faeces, & Salmonella [15].

When colitis and/or bloody stools are present, it is essential to check for Clostridium difficile. It's crucial to remember that people without a history of antibiotic usage can still get acute-onset diarrhoea caused by a C. difficile infection. One should identify the kind of E coli in instances of diarrhoea if consuming ground beef during enterohemorrhagic E coli outbreaks has a history has been identified on the settee because E coli O157:H7 infection can induce hemolytic uremic syndrome [16]. Enzyme immunoassay & latex agglutination from faeces are used to detect rotavirus antigen. Adenovirus antigens can be discovered using an enzyme immunoassay. The best technique to identify parasites is to examine your stool for ova and parasites. Every three days, or every other day, the stool test should be done [17].

2. EXPERIMENTAL

2.1. Research Design

A cross-sectional study was conducted by our hospital from September 2022 to August 2023. To conduct this study, stool samples were collected and tested for RVA by quantities of RT-PCR. The stool samples were collected from asymptomatic children who were under five years old as well as those who had diarrhea or a record of diarrhea within 24 hours. In addition, the sample was selected based on the RDT analysis performed and the predominant strains among the RVA RT-qPCR positives. The stool sample was tested by using a commercially available RDT. Furthermore, the kit manufacturer using a swab transferred a small amount of stool sample. At least these times, the Yep suspension was homogenized by swirling. As per the instruction, the result of the Te test was read after 20 min. On the other hand, viral RNA was extracted from 140 µl of stool suspension and subsequently RNA using QIAamp viral RNA Mini Kit. The RNA isolation was performed by manufacturer instructions such as the genomic RNA was eluted in a volume of 60 µl and stored at –80 °C. By using a single-step RT-qPCR protocol targeting the NSP4 gene, the presence of RVA was confirmed. For diluted and denatured at 95°C for 1 min, RT- qPCR, the RNA was extracted in the total volume of 12 µl by using the SuperScript III/Platinum Taq OneStep kit. Again, samples with a cycle threshold (Ct) ≤39 were considered as positive as well and it was subsequently genotyped based on the amplification of the VP7 and VP4 genes. After evaluating the presence and absence of RVS gastroenteritis, the cut of the value of CT was 24.

Inclusion criteria

- Under five years of age.
- Children with diarrhea or record of diarrhea within 24 h.
- The stool sample was collected from asymptomatic children who were under 5.

Exclusion criteria

- More than 5-year-old children were excluded from this study.
- Patients who had no record of diarrhea were excluded from this study.

2.2. Statistical analysis

Byusingthe GraphPad Prism 6.00, this study was performed. The value was calculated by evaluating the performance of the SD BIOLINE Rota and Adeno Ag RDT. Furthermore, the comparison of RT-qPCR is defined to the reference method. In addition, Te test performance is measured by presenting the percentages with their respective 95% confidence interval (CI). Even Ct value allotment between diarrhea cases and asymptomatic controls was tested by using the Mann-Whitney test. Moreover, this study used Chi-squared tests and the p-value was <0.05 which was considered statistically significant.

3. RESULTS

The baseline characteristics of individuals with or without diarrhoea are shown in Table 1. Each group has 41 patients in the trial. Patients with diarrhoea average 10.95 months old, while those without diarrhoea are 11.56 months old. Male patients make up 56.09% of the diarrhoea group and 53.65% of the no-diarrhea group. Urban residents make up 43.90% of the diarrhoea group and 39.02% of the no-diarrhea group, while semi-urban and rural residents make up the rest. This table shows the demographic and residential features of the patient population,

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which may help explain any associations with diarrhoea in the study.

Table 1: Baseline characteristics of the patients grouped based on presence of diarrhea

Parameters	Presence of diarrhea n=41	No diarrhea n=41
Age (months)	10.95	11.56
Sex		
Male; n (%)	23 (56.09%)	22 (53.65%)
Female; n (%)	18 (43.90%)	19 (46.34%)
Live in		
Urban, n (%)	18 (43.90%)	16 (39.02%)
Semi-urban	11 (26.82%)	12 (29.26%)
Rural	12 (29.26%)	13 (31.70%)

In Figure 1, the number of patients with diarrhoea and high Cycle Threshold (Ct) values is compared to Positive RT-qPCR and Positive Rapid Diagnostic Test results. Patients experiencing fever, vomiting, gastroenteritis, muscle discomfort, and respiratory tract infection are listed in the table. Of the 41 diarrhoea patients, 25 experienced fever, 25 vomiting, 25 gastroenteritis, 25 muscle discomforts, and 23 RTI. Similar to the 41 high Ct patients, symptom counts are listed. Additionally, the data indicates the number of patients with each symptom among the 43 positive RT-qPCR results and the 18 positive RDT results. This data permits in-depth examination of clinical aspects related with different parameters and test results, revealing illness insights for further study.

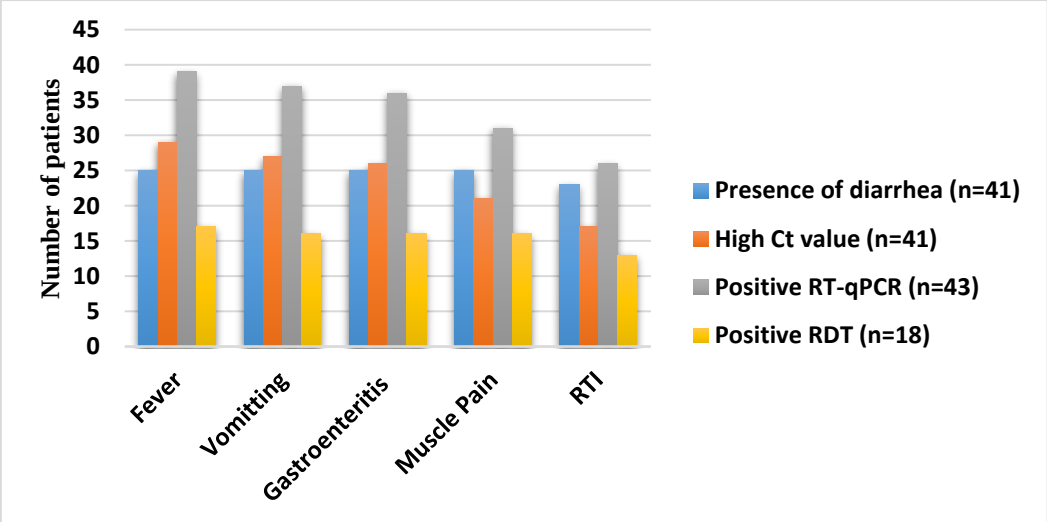

Figure 1: Number of patients with each of the clinical features with each of the parameters

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Table 2 compares RT-qPCR and RDT results for virus strain identification. Sample sizes are listed in brackets for G12P, G1P, and G8P strains. RT-qPCR detected 62.79% of G12P instances, while RDT identified 50%. On the G1P strain, RT-qPCR exhibited a 20.93% success rate compared to RDT's 33.34%. RT-qPCR and RDT both identified 16.27% and 16.67% of G8P cases, respectively. In general, RT-qPCR beats RDT in detecting these viral strains, however it depends on the strain.

Table 2: Strains identified by each of the method (RT-qPCR and RDT)

Strain Identified	Positive RT-qPCR (Total=43); n (%)	Positive RDT (Total=18); n (%)
G12P (n=27)	27 (62.79%)	9 (50%)
G1P (n=9)	9 (20.93%)	6 (33.34%)
G8P (n=7)	7 (16.27%)	3 (16.67%)

The Rapid Diagnostic Test (RDT) and RT-qPCR diagnostic properties are shown in Figure 2. Compared to the gold standard RT-qPCR, the table shows how well the RDT identifies positive and negative cases. The RDT's sensitivity was 41.86% since 18 of those with a positive RDT also had a positive RT-qPCR. Among those with a negative RDT result, 39 had a negative RT-qPCR result, indicating the RDT's ability to identify true negatives. RDTs with positive results are highly dependable, since their Positive Predictive Value (PPV) is 100.00%. Since the Negative Predictive Value (NPV) is 60.94%, the RDT is likely right when it returns a negative result. Compared to the RT-qPCR gold standard, the RDT classifies cases with 69.51% accuracy.

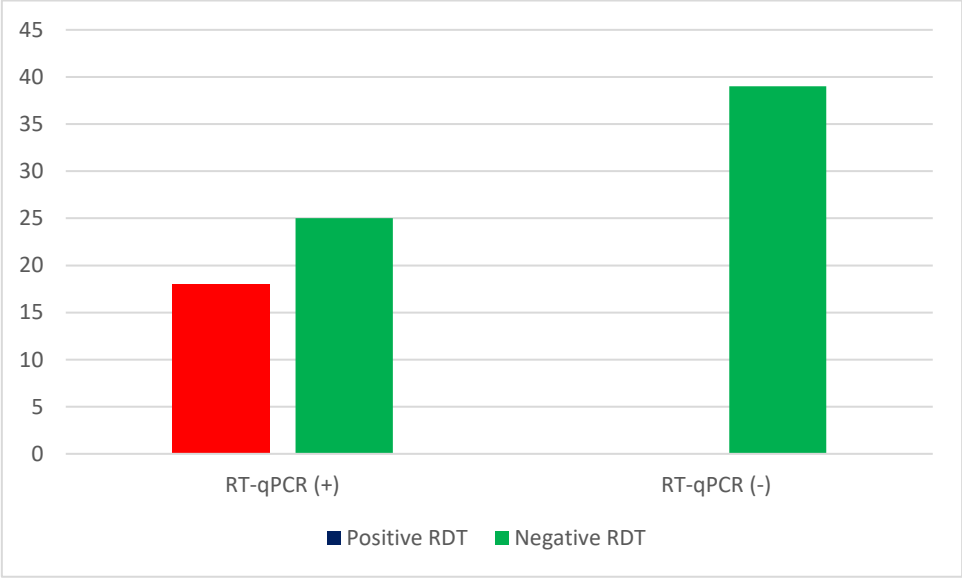

Figure 2: Diagnostic Characteristics of RDT with respect to RT-qPCR

The Rapid Diagnostic Test (RDT)'s ability to identify Cycle Threshold (Ct) values for a parameter is shown in Table 3. Six samples with low Ct levels were positive, while 12 of 41 with high Ct values were correct. The RDT has 14.63% sensitivity for low Ct levels. However, its specificity of 70.73% detects high Ct values. PPV = 33.33%, indicating that a positive RDT result increases the likelihood of

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a low Ct value. If the RDT returns a negative result, the Ct value is 45.31% likely to be high. The RDT accurately locates Ct values 42.68% of the time. The RDT is superior at detecting high Ct values, but its sensitivity for low Ct values is restricted.

Table 3: Validity of RDT in identifying Ct value

Parameters	Positive RDT	Negative RDT
Low Ct value	6	35
High Ct value	12	29
Sensitivity	14.63%	
Specificity	70.73%	
PPV	33.33%	
NPV	45.31%	
Accuracy	42.68%	

Table 4 compares Rapid Diagnostic Test (RDT) findings to the gold standard RT-qPCR test for diarrhoea detection. The RDT correctly detected symptomatic diarrhoea cases with 52.00% sensitivity. However, its sensitivity lowers to 27.78% for asymptomatic cases without diarrhoea, indicating inferior accuracy. However, the specificity, PPV, and NPV for both categories are 100.00%, demonstrating that the RDT is highly accurate and trustworthy when it produces a positive or negative result. The RDT accurately detects diarrheal cases 70.73% of the time and non-diarrhea patients 68.29%. These data imply that the RDT is better at identifying symptomatic diarrhoea cases but less reliable at identifying asymptomatic ones.

Table 4: Validity of RDT in identifying symptomatic and asymptomatic cases

Parameter	Presence of diarrhea		No diarrhea	
RDT Result	Positive	Negative	Positive	Negative
RT-qPCR (+)	13	12	5	13
RT-qPCR (-)	0	16	0	23
Sensitivity	52.00%		27.78%	
Specificity	100.00%		100.00%	
PPV	100.00%		100.00%	
NPV	57.14%		63.89%	
Accuracy	70.73%		68.29%	

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Figure 3 shows the number of positive and negative Rapid Diagnostic Test (RDT) and RT-qPCR results. Among those who tested positive with RT-qPCR (a gold standard for detection), 18 also tested positive with the RDT, giving a sensitivity of 41.86%, suggesting that the RDT may have some limits in recognising true positives. However, it had 100% specificity, meaning all RT-qPCR negatives were RDT negative. When the RDT returns a positive result, the PPV is 100.00%, indicating great reliability. When the RDT returns a negative result, it has a 60.94% chance of being right. RDT accuracy is 69.51%, indicating that it is highly specific but less sensitive, especially in identifying true positive cases.

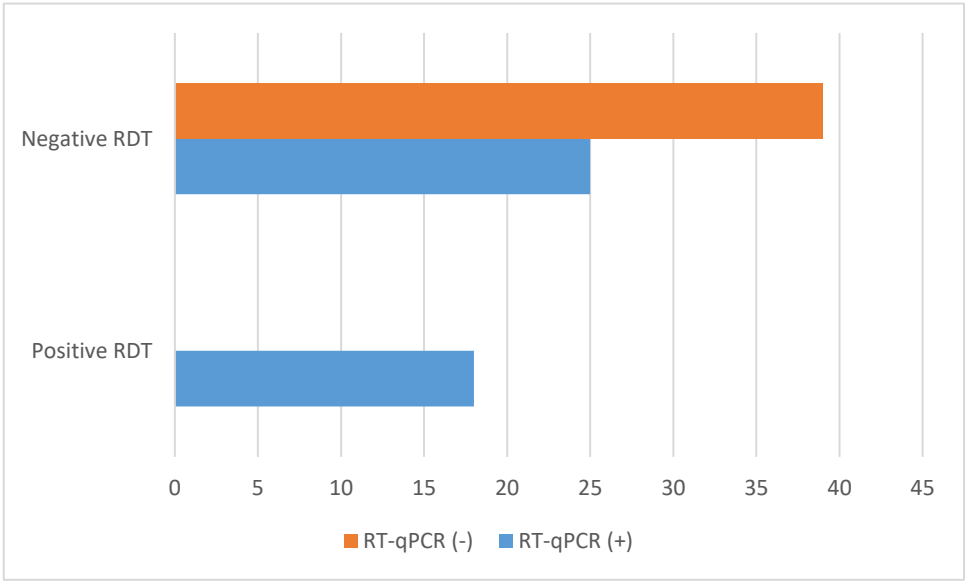

Figure 3: Number of cases of positive and negative RDT with that of positive and negative cases of Rt-qPCR. Sensitivity 41.86%; Specificity 100.00%; PPV 100.00%; NPV 60.94%; Accuracy 69.51%.

4. DISCUSSION

Children all across the world continue to be affected by severe acute diarrhoea caused by rotavirus A (RVA) infections. Nowadays, RVA is routinely identified with rapid diagnostic tests (RDT). Pediatricians are, however, dubious of the RDT's continued accuracy in viral detection [18]. Therefore, the intention of the research was to contrast the efficacy of the one-step RT-qPCR technique with the quick rotavirus test. From April 2018 until November 2019, a cross-sectional research was undertaken in Lambaréné, Gabon [19]. Children under the age of five a 24-hour period of diarrhoea or a bout of diarrhoea, as well as asymptomatic kids stool samples were collected from similar communities [20]. The gold-standard qualitative reversed transcription-q PCR (RT-qPCR) has been used to produce and analyse each and every stool sample in comparison to the RDT. Even though RT-qPCR missed a lot of asymptomatic RVA cases, this RDT showed excellent sensitivity and was sufficient for identifying RVA in patients with RVA gastroenteritis. It could be a helpful diagnostic tool, particularly in developing nations with poor incomes [21].

The purpose of the present research was to contrast the results of two readily accessible kits used for routine Rotavirus A identification in specimens of human faeces to results from internal RT-qPCR and commercial RT-qPCR testing. RT-qPCR was more sensitive than commonly used EIA or RDT techniques for monitoring rotavirus gastroenteritis. Both of the analysed tests have very high specificities. However, EIA was evaluated as having superior performance than RDT across the board [22].

Despite the widespread use of rotavirus vaccinations (RotaTeq/Rotarix/ROTAVAC/Rotasiil), group A rotavirus (RVA) still causes 104,000 fatalities and 600,000 hospital admissions per year in sub-Saharan Africa. RotarixTM was released in Cameroon in

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March 2014, although the effects of the vaccine's introduction have not yet been studied. Routine laboratory testing of rotavirus infection has yet to become a widespread practice. Therefore, research on RVA prevalence following vaccine introduction is required [23]. The study's objectives were to determine the prevalence of RVA in instances of severe diarrhoea within the Littoral region of Cameroon and to look at the role of other bacteria that cause diarrhoea in RVA-positive patients. It reveals greater than anticipated RVA prevalence across vaccinated children admitted to hospitals for diarrhoea, offers information on the frequency of RVA across Cameroon that might be useful for epidemiological research conducted after vaccination and demonstrates a propensity for RVA co-infection with other enteric infections. RVA genotyping is necessary to detect circulating rotavirus genotypes in Cameroon, particularly ones causing illness in immunised children.

Children all across the world continue to be affected by severe acute diarrhoea caused by rotavirus A (RVA) infections [24]. Nowadays, RVA is routinely identified with rapid diagnostic tests (RDT). Paediatricians, however, are sceptical about the RDT's continued accuracy in viral detection. Therefore, Its objective was to contrast the efficacy of the one-step RT-qPCR technique with the quick rotavirus test. Although most symptomatic While RT-qPCR failed to detect RVA shedding, this RDT showed excellent sensitivity and was sufficient for identifying RVA in patients with RVA gastroenteritis. It could be a helpful diagnostic tool, particularly in developing nations with poor incomes [25].

In the study, stool samples from kids who Comparing and evaluating the specificity & sensitivity of three immunochromatography (IC) kits employed for the rapid diagnosis of group A rotavirus in patients who experienced acute gastroenteritis during February to June 2009 in Japan. A reference RT-PCR technique was used to examine and compare an aggregate of 86 stool samples [26]. Test kits for IP-Rota V, Dipstick Eiken ROTA, and ROTA-ADENO had sensitivity of 97.2, 95.8, and 88.7%, respectively, while their specificities were 100, 93.3, and 100%. It was shown that, particularly during the season of acute gastroenteritis outbreaks, the IC kits examined in this study might be employed as a substitute approach for the rapid detection of group A rotavirus in faecal materials [27].

Rotavirus A is usually detected by rapid testing; however paediatricians are more often sceptical about how well the tests still work. The one-step RT-PCR technique was used in this study's evaluation of this rotavirus rapid test's performance. Children who had serious diarrhoea had their stool samples taken from 755 of them. All samples were handled as soon as they arrived using the one-step RT-PCR procedure and the SD BIOLINE rota fast test. Although the fast test may produce findings rapidly, we discovered that it has significant rates of false positive and false negative outcomes. Therefore, additional extremely sensitive techniques like one-step RT-PCR remain required for accurate diagnosis [28].

Since diarrhoeaIdentification of the infection as soon as possible and with accuracy is crucial since it is the second-most prevalent cause of mortality in children below the age of five in patients who have diarrhoea in order to lower morbidity and mortality [29]. The Allplex GI-Virus Assay, a recently created multiplex real-time PCR test in one step, was tested to see if it could identify six viruses that might cause diarrhoea in stool samples: rotavirus, enteric adenovirus, astrovirus, and sapovirus are some of the common noroviruses. Using the Allplex test, able to detect sapoviruses in addition to showing strong agreement to Seeplex and genotyping findings. Patients experiencing acute gastroenteritis signs may benefit from the Allplex assay to identify gastrointestinal viral infections [30].

5. CONCLUSION

The study has concluded that the accuracy of designed RDT is about 70% in the presence of diarrhea while it is 68% in the absence of diarrhea. Again, it is much less accurate in identifying higher Ct value. However, this RDT can be used to identify rotavirus infection and to rule out or consider rotavirus infection clinically. The study is limited to less number of cases and broader study should be conducted in the future after certain modification or improving the RDT. Although RT-qPCR remains the ultimate diagnostic tool, this design RDT can be

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used by common people at an economic price in ruling out rotavirus A.

This study has employed RT-PCR and Ct values as benchmarks, demonstrating the potential of this newly designed RDT. The results revealed promising specificity in RVA detection, indicating its potential as an affordable and accessible diagnostic tool. However, it exhibited lower sensitivity compared to RT-qPCR. This underscores the need for further refinement and validation of the RDT. Overall, this study highlights the importance of accessible diagnostic tools in managing infant diarrheal cases, particularly in resource-limited settings, where timely identification of RVA is critical for effective clinical intervention.

REFERENCES

[1] Chen J, Wan CM, Gong ST, Fang F, Sun M, Qian Y, Huang Y, Wang BX, Xu CD, Ye LY, Dong M, Jin Y, Huang ZH, Wu QB, Zhu CM, Fang YH, Zhu QR, Dong YS. Chinese clinical practice guidelines for acute infectious diarrhea in children. World J Pediatr. 2018 Oct;14(5):429-436.

[2] Null C, Stewart CP, Pickering AJ, Dentz HN, Arnold BF, Arnold CD, Benjamin-Chung J, Clasen T, Dewey KG, Fernald LCH, Hubbard AE, Kariger P, Lin A, Luby SP, Mertens A, Njenga SM, Nyambane G, Ram PK, Colford JM. Effects of water quality, sanitation, handwashing, and nutritional interventions on diarrhoea and child growth in rural Kenya: a cluster-randomised controlled trial. Lancet Glob Health. 2018 Mar;6(3):e316-e329.

[3] Wenzl HH. Diarrhea in chronic inflammatory bowel diseases. GastroenterolClin North Am. 2012 Sep;41(3):651-75.

[4] Jiang ZD, DuPont HL. Etiology of travellers' diarrhea. J Travel Med. 2017 Apr 01;24(suppl_1):S13-S16.

[5] Hoelzer K, Moreno Switt AI, Wiedmann M. Animal contact as a source of human non-typhoidal salmonellosis. Vet Res. 2011 Feb 14;42(1):34.

[6] Crawford SE, Ramani S, Tate JE, Parashar UD, Svensson L, Hagbom M, Franco MA, Greenberg HB, O'ryan M, Kang G, et al. Rotavirus infection HHS public access. JAMA Pediatr. 2018;172:503.

[7] Matthijnssens J, Ciarlet M, Heiman E, Arijs I, Delbeke T, McDonald SM, et al. Full genome-based classification of rotaviruses reveals a common origin between human wa-like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. J Virol. 2008;82(7):3204–19.

[8] Matthijnssens J, Heylen E, Zeller M, Rahman M, Lemey P, Van Ranst M. Phylodynamic analyses of rotavirus genotypes G9 and G12 underscore their potential for swift global spread. MolBiolEvol. 2010;27(10):2431–6.

[9] Troeger C, Khalil IA, Rao PC, Cao S, Blacker BF, Ahmed T, et al. Rotavirus vaccination and the global burden of rotavirus diarrhea among children younger than 5 years. JAMA Pediatr. 2018;172(10):958–65.

[10] Soltan MA, Tsai YL, Lee PYA, Tsai CF, Chang HFG, Wang HTT, Wilkes RP. Comparison of electron microscopy, ELISA, real time RT-PCR and insulated isothermal RT-PCR for the detection of Rotavirus group A (RVA) in feces of different animal species. J Virol Methods. 2016;235:99–104.

[11] Yolken RH, Kim HW, Clem T, Wyatt RG, Kalica AR, Chanock RM, Kapikian A. Enzyme-linked immunosorbent assay (ELISA) for detection of human reovirus-like agent of infantile gastroenteritis. Lancet. 1977;2:263–7.

[12] Nikfarjam M, Wilson JS, Smith RC., Australasian Pancreatic Club Pancreatic Enzyme Replacement Therapy Guidelines Working Group. Diagnosis and management of pancreatic exocrine insufficiency. Med J Aust. 2017 Aug 21;207(4):161-165.

[13] Ethelberg S, Olesen B, Neimann J, Schiellerup P, Helms M, Jensen C, Böttiger B, Olsen KE, Scheutz F, Gerner-Smidt P, Mølbak K. Risk factors for diarrhea among children in an industrialized country. Epidemiology. 2006 Jan;17(1):24-30.

[14] Costard S, Espejo L, Groenendaal H, Zagmutt FJ. Outbreak-Related Disease Burden Associated with Consumption of Unpasteurized Cow's Milk and Cheese, United States, 2009-2014. Emerg Infect Dis. 2017 Jun;23(6):957-964.

[15] Rosner BM, Stark K, Höhle M, Werber D. Risk factors for sporadic Yersinia enterocolitica infections, Germany 2009-2010. Epidemiol Infect. 2012 Oct;140(10):1738-47.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(3), 1114-1123 | ISSN:2251-6727

[16] Manouana GP, Nguema-Moure PA, Tomazatos A, Maloum MN, Bock CT, Kremsner PG, Velavan TP, Adegnika AA, Niendorf S. RT-PCR-based assessment of the SD Bioline Rota/Adeno Antigen-based test in infants with and without diarrhea. Virol J. 2023 Mar 3;20(1):40.

[17] Moutelíková R, DvořákováHeroldová M, Holá V, Sauer P, Prodělalová J. Human rotavirus A detection: Comparison of enzymatic immunoassay and rapid chromatographic test with two quantitative RT-PCR assays. EpidemiolMikrobiolImunol. 2018 Winter;67(3):110-113.

[18] Ghapoutsa RN, Boda M, Gautam R, Ndze VN, Mugyia AE, Etoa FX, Bowen MD, Esona MD. Detection of diarrhoea associated rotavirus and co-infection with diarrhoeagenic pathogens in the Littoral region of Cameroon using ELISA, RT-PCR and LuminexxTAG GPP assays. BMC Infect Dis. 2021 Jun 28;21(1):614.

[19] Manouana GP, Niendorf S, Tomazatos A, MbongNgwese M, NzambaMaloum M, Nguema Moure PA, BingoulouMatsougou G, Ategbo S, Rossatanga EG, Bock CT, et al. Molecular surveillance and genetic divergence of rotavirus A antigenic epitopes in Gabonese children with acute gastroenteritis. EBioMedicine. 2021.

[20] Japhet MO, Famurewa O, Iturriza-Gomara M, Adesina OA, Opaleye OO, Niendorf S, Bock CT, Mas Marques A. Group A rotaviruses circulating prior to a national immunization programme in Nigeria: clinical manifestations, high G12P[8] frequency, intra-genotypic divergence of VP4 and VP7. J Med Virol. 2018;90:239–49.

[21] Corcoran MS, van Well GTJ, van Loo IHM. Diagnosis of viral gastroenteritis in children: interpretation of real-time PCR results and relation to clinical symptoms. Eur J ClinMicrobiol Infect Dis. 2014;33:1663–73.

[22] Phillips G, Lopman B, Tam CC, Iturriza-Gomara M, Brown D, Gray J. Diagnosing rotavirus A associated IID: using ELISA to identify a cut-off for real time RT-PCR. J ClinVirol. 2009;44:242–5.

[23] Chieochansin T, Vutithanachot V, Theamboonlers A, Poovorawan Y. Evaluation of the rapid test for human rotavirus a in Thai children with acute gastroenteritis. Clin Lab. 2014;60:511–4.

[24] Tate JE, Mijatovic-Rustempasic S, Tam KI, Lyde FC, Payne DC, Szilagyi P, Edwards K, Staat MA, Weinberg GA, Hall CB, et al. Comparison of 2 assays for diagnosing rotavirus and evaluating vaccine effectiveness in children with gastroenteritis. Emerg Infect Dis. 2013;19:1245–52.

[25] Phillips G, Lopman B, Rodrigues LC, Tam CC. Asymptomatic rotavirus infections in England: Prevalence, characteristics, and risk factors. Am J Epidemiol. 2010;171:1023–30.

[26] Elliott EJ. Acute gastroenteritis in children. *BMJ*. 2007; **334**(7583): 35–40.

[27] Troeger C, Khalil IA, Rao PC, Cao S, Blacker BF, Ahmed T, et al. Rotavirus vaccination and the global burden of rotavirus diarrhea among children younger than 5 years. *JAMA Pediatr*. 2018; **172**(10): 958–65.

[28] Khamrin P, Tran DN, Chan-it W, Thongprachum A, Okitsu S, Maneekarn N, Ushijima H. Comparison of the rapid methods for screening of group a rotavirus in stool samples. J Trop Pediatr. 2011 Oct;57(5):375-7. doi: 10.1093/tropej/fmq101. Epub 2010 Oct 28. PMID: 21030457.

[29] Chieochansin T, Vutithanachot V, Theamboonlers A, Poovorawan Y. Evaluation of the rapid test for human rotavirus A in Thai children with acute gastroenteritis. Clin Lab. 2014;60(3):511-4. doi: 10.7754/clin.lab.2013.130616. PMID: 24697131.

[30] Hyun J, Ko DH, Lee SK, Kim HS, Kim JS, Song W, Kim HS. Evaluation of a New Multiplex Real-Time PCR Assay for Detecting Gastroenteritis-Causing Viruses in Stool Samples. Ann Lab Med. 2018 May;38(3):220-225.