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Evaluation and Validation of four Rapid Diagnostic test (RDTs) kits available for SARS-CoV-2 Detection in Rivers State, Nigeria

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Abstract

Overview: Due to the exponential rise in testing demand, Reverse transcription real time Polymerase chain (Rt-PCR) reaction the gold standard used to investigate Covid-19 is beset for material shortages, high costs, and lengthy turnaround times. The novel Coronavirus Rapid detection kits are employed in place of RT-PCR due to their reduced costs, quicker turnaround times, and lower prices but there are numerous worries about their specificity and sensitivity.

Objectives: We evaluated and validated the analytic performance of four rapid antigen diagnostic test kits (RDTs) I-4 SD Biosensor (RDT1), Abbott (RDT2), Biobase (RDT3) and Dia Pro (RDT4) in use in Rivers State Nigeria for the identification of COVID-19 built on its exactitude and responsiveness compared with the cycle threshold (CT) of the benchmark of diagnosis quantitative reverse transcription PCR.

Methods: A comprehensive study was conducted in Rivers State, involving 3705 individuals of both genders, aged 20 to 65, presenting symptoms or not, at three prominent COVID-19 NCDC-accredited centers. The aim was to assess the effectiveness and precision of four rapid diagnostic test kits for detecting SARS-COV-2, compared to the cycle threshold of the gold standard diagnostic method, reverse transcriptase real-time polymerase chain reaction (Rt-PCR). All tests were conducted following the manufacturer's guidelines within a biosafety cabinet of class 2 level.

Result: Out of 3705 samples enrolled in the diagnostic accuracy analysis, 201 with Cycle threshold value (Ct) of ≤ 40 as the cut-off was positive for the virus with RtPCR giving a frequency rate of 5.4%. At Ct value ≤ 30 and CT > 30 for ORF gene, N-GENE and E-GENE, Abbott gave a total number of 151 positive results followed by Standard Q (120) and then Biobase (72). The least positive result was obtained with Diapro (36). With the RDTs prevalence rate of 3.3% was obtained. All RDTs showed 100.0% Specificity. Abbott has the highest sensitivity rate of 75.1% followed by Standard Q (59.7%) and then Biobase (35.8%). The least was seen with Dia.Pro (17.9%). All RDTs gave PPV of 100.0% and NPV Abbott (98.0%), Standard Q (97.7%), Biobase (96.4%) and Dia.Pro (95.5%).

Conclusions: The rapid Antigen tests showed promising sensitivity in identifying individuals with lower Ct values, though it's crucial to better understand their correlation with infectivity. The Abbott (RDT2) test demonstrated notable specificity and sensitivity, particularly for diagnosing SARS-CoV-2 when Ct values are below 30. This implies its potential value in frontline testing

compared to conventional rRT-PCR methods which serve as the benchmark especially when PGR isn't immediately accessible. NCDC recommended the Abbott rapid diagnostic test kit.

Keywords: COVID-19, SARS-CoV-2, RDTs, Rt-PCR, Standard Q, Abbott, Biobase, Dia.Pro

Definition of terms

In this context, we used several abbreviations: TP for True Positive, FN for False Negative, FP for False Positive, and TN for True Negative. Sensitivity, which indicates the accuracy of identifying positive cases, was calculated as the percentage of samples that tested positive with the NP antigen testing assay among those that were actually positive ($\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN}) \times 100$). Specificity, indicating the precision in identifying negative cases, was determined as the percentage of samples that tested negative with the NP antigen testing assay among those that were truly negative ($\text{Specificity} = \text{TN} / (\text{FP} + \text{TN}) \times 100$). Positive predictive value (PPV) represented the likelihood that a positive test result truly indicated infection, calculated as the percentage of true positives among all positive results ($\text{PPV} = \text{TP} / (\text{TP} + \text{FP}) \times 100$). Negative predictive value (NPV) represented the likelihood that a negative test result accurately indicated the absence of infection, calculated as the percentage of true negatives among all negative results ($\text{NPV} = \text{TN} / (\text{FN} + \text{TN}) \times 100$). Additionally, Ct refers to Cycle threshold, a measure used in PCR testing.

1. INTRODUCTION

COVID-19 virus, was first seen in in Wuhan, China's Hubei Province in December 2019. It has spread to more than 200 nations, posing enormous difficulty to worldwide healthcare systems resulting in thousands of fatalities. It has grown to be a global public health emergency. In various parts of the world, a deficiency in widespread testing and treatment strategy has hindered control and prevention initiatives, leading to a high counts of cases and mortality, primarily in nations with low to middle incomes, such as Nigeria., which is one of the main causes for the grave situation brought about by the COVID-19 pandemic (Torres & Sacoto, 2020; Henriquez-Trujillo et al., 2021; Pullano et al., 2021).

The objectives of any containment strategy encompass managing, preventing, and promptly diagnosing SARS-CoV-2 which is Crucial for both management and containment of the virus. Novel ways to significantly improve diagnostic capabilities are urgently warranted due to the rapidly rising incidence of COVID-19 infections (Sharfstein et al., 2020). The standard way for the detection of SARS-COV-2 is by amplification of virus nucleic acids Using reverse transcription real-time PCR (rRT-PCR) (WHO, 2020; CDC, 2020). Nevertheless, using the Rt-PCR test for community screening is challenging due to its complexity (Mina et al., 2021). These viruses or another kind, comparable to how SARS-COV-1 and SARS-COV-2 have resurfaced, will probably reappear. Therefore, the first step towards treatment and quarantine is a prompt and correct diagnosis of the SARS-CoV-2 infection.

A test that isn't easily accessible or available for routine use may not be as effective as a monitoring routine aimed at containing the spread of viruses (Mina et al., 2020). The difficulties of RT PCR include expensive laboratory reagents and equipment, continuous power supplies, specialist laboratory staff, and lengthy turnaround times, this eventually prevents the testing capacity from being scaled up, necessitating the use of different testing formats. Once more, a rapid and brief turn-around test is required to slow and manage the virus's transmission to prompt patient monitoring, isolation, treatment, and population screening to stop the virus from spreading further, especially in low-income countries like Nigeria (Lai et al., 2020). Rapid Antigen diagnostic test kits

(RDTs) meet all these criteria and represent one such alternate diagnostic technique but are less sensitive compared to the standard RtPCR.

Rapid Diagnostic Tests (RDTs) are swift, inexpensive, dependable, accurate, and straightforward identification tests that do not necessitate sophisticated equipment or reagents. They can be conducted in the field without the need for electricity. Rapid antigen-based diagnostic assays are readily available and could help with testing in home and community settings, hasten the start of treatment and maximize the time spent in isolation (Peeling et al., 2021; Drain, 2022). Without the need for expensive equipment, this test can identify SARS-CoV-2 infection in 10 minutes. Significantly, accelerating the process from testing to receiving results can speed up the isolation of contagious individuals, which is particularly vital in areas with high transmission rates, where widespread testing is imperative. Although antigen tests may have lower sensitivity in detecting infections compared to real-time Rt-PCR, they still offer swift identification, leading to quicker isolation and better outbreak management.

Reverse transcription-polymerase chain reaction can identify trace levels of SARS-CoV-2 nucleic acid fragments which can continue to be positive long after recovery (Mallett et al., 2020). Because of this, the CDC do not advise utilizing an approach based on RT-PCR testing to conclude hospital seclusion for the majority of patients (USCDC, 2022). In comparison to a more sensitive test that is less accessible, a less sensitive test that is more widely available might detect more cases. Once more, early on in an infection, immunoglobulin reaction to a pathogen do not manifest. Consequently, early infestation detection cannot be achieved by antibody-based testing.

One promising approach for identifying SARS-CoV-2 infection early on is through the detection of viral antigens. The nucleocapsid protein (NP) is among the structural proteins frequently expressed, making it a compelling candidate for early detection in SARS-CoV infection. Since SARS-CoV, which emerged in 2003, shares significant genetic similarities with SARS-CoV-2, the NP antigen is particularly notable as one of the most reliable early markers for detecting SARS-CoV-2, detectable up to a day before clinical symptoms manifest (Wu et al., 2020).

In most communities in Nigeria during the pandemic, Samples are collected and sent to cities. Many people die waiting for Rt-PCR results. RDT an alternative to Rt-PCR is a prerequisite for now and for the future in case of another pandemic. Numerous distributors have pushed several commercially available rapid diagnostic tests using antigen detection (Ag-RDTs) onto the market. Leaving potential users uninformed without significant variation regarding their diagnostic effectiveness and external validation (Mattiuzzi et al., 2020). However, the reliability, sensitivity and precision of this diagnostic approach should be examined (CDC, 2020: Smith et al., 2021).

The aim of this study is to evaluate and validate the reliability and consistency of four rapid antigen diagnostic test (RDT) kits in identifying SARS-CoV-2. The assessment involves comparing their effectiveness with the established Reverse Transcriptase Real-Time Polymerase Chain Reaction (RT-PCR) technique, regarded as the benchmark. Both asymptomatic and symptomatic individuals in Rivers State, Nigeria, are included in the investigation, with samples collected from nasopharyngeal and oropharyngeal swabs.

2. MATERIALS AND METHOD

2.1. Study area

The research was carried out in three key NCDC COVID-19 accredited facilities located in Rivers State, Nigeria: Rivers State University Teaching Hospital Rivers State, University of Port Harcourt COVID-19 Laboratory and University of Port Harcourt Teaching Hospital situated in the southern

region of Nigeria's Niger Delta, holds historical significance, having been part of the former Eastern Region before gaining autonomy in 1967. Geographically, it shares borders with Imo and Anambra to the north, Abia and Akwa Ibom to the east, and Bayelsa and Delta to the south, with the Atlantic Ocean marking its southern limit. Its landscape ranges from level plains crisscrossed by numerous rivers to tributaries. The state's population was 5,198,716 according to the 2006 census and had increased to 7,492,366 by 2023, making it the seventh most populous state in Nigeria.



Figure 1:Map of Rivers State (Nigeria)

2.2. Study design

This study aims to evaluate the diagnostic accuracy of four rapid diagnostic test kits for detecting SARS-CoV-2 using nasopharyngeal and oropharyngeal swab samples. These tests will be compared with the gold standard method of reverse transcriptase real-time polymerase chain reaction (RT-PCR). The study will involve symptomatic and asymptomatic individuals suspected of having COVID-19, drawn from three COVID-19 centers in Rivers State, covering the period between 2021 and 2022.

2.3. Ethical Considerations and Approval:

The study obtained ethical approval from Rivers State University Teaching Hospital under reference RUSTH/REC/2022190 and from the University of Port Harcourt under reference UPH/CEREMAD/REC/MM88/056. Additionally, participants provided consent before the administration of questionnaires.

2.4. Inclusion and Exclusion Criteria

This study encompassed individuals aged between 20 and 70 years, both male and female, whether they displayed symptoms of the disease or not. However, individuals aged 0 to 19 years, nursing mothers, and pregnant women were excluded from participation in the study.

2.5. Sample collection

A total of 3705 samples were collected from individuals suspected of having COVID-19, whether they showed symptoms or not, at three Nigeria Center for Disease Control and Prevention (NCDC) accredited centers in Rivers State, Nigeria. The distribution of samples collected from each center was as follows: 387 (10.4%) from the University of Port Harcourt Teaching Hospital, 2728 (73.6%)

from the University of Port Harcourt COVID-19 laboratory, and 590 (15.9%) from Rivers State University Teaching Hospital.

For each participant, two swabs, one from the nasopharynx and one from the oropharynx, were collected and transported to the laboratory while maintaining a cold chain. Swabs designated for antigen testing underwent analysis according to the manufacturer's instructions, while those for real-time RT-PCR were stored in viral transport media (VTM) at 39°F (4°C) and analysed within 24–72 hours of collection. Both Nucleic Acid (NA) testing using Reverse Transcriptase Real-Time Polymerase Chain Reaction (RT-PCR) and Nucleocapsid Protein (NP) antigen testing using four Rapid Diagnostic Antigen test kits were conducted in a level 2 biosafety cabinet.

Participants provided demographic information through a questionnaire approved by the NCDC. This comprehensive approach aimed to gather data on a diverse range of individuals to assess the diagnostic accuracy of the testing methods employed.

2.6. Molecular Analysis using Rt-PCR

Real-Time PCR (RT-PCR) stands as the premier method for diagnosing SARS-CoV-2, a method which involves the use of precise primers to detect viral RNA by targeting the ORF gene, E gene and N gene in the SARS-CoV-2 genome using Real Time Multiplex RT-PCR Coronavirus Kit (Liferiver[®], Shanghai China). Identification of N-gene, ORF gene and E-gene based on the Amplification cycle threshold (Ct) cut off value of <40 are factors of positive SARS-COV-2 sample (Tang et al., 2020; Kevadiya et al., 2021). Protocol for Real-Time Polymerase Chain Reaction (RT-PCR) include;

2.6.1. Inactivation of sample

Wearing the proper personal protective equipment (PPE), the inactivation procedure Was conducted in a Level 2 Biosafety cabinet (BSL-2) with the Addprep Nucleic Acid Viral Extraction Kit, Korea, in accordance following the manufacturer's instructions. Using a Micropipette 200ul of Sample was placed in 350ul of lysis buffer containing 3.5µl β-Mercaptoethanol in a 1.5ml centrifuge tube and mixed by pulse vortexing for 10-15 sec (Enitan, 2020). The mixture was incubated for 10 min and 150µl of absolute ethanol was added. mixed well by pulse-vortexing for 5 seconds. It was spun briefly to get the drops clinging under the lid. A nuclease-free water in a separate tube was used as Negative Extraction Control (NEC) to monitor the extraction process. This was treated with the same procedure alongside with the sample. After sample inactivation, Personal Protective Equipment was doffed and other materials used was sanitized, autoclaved and disposed according to waste management guideline.

2.6.2. Extraction /Elution of SARS-CoV-2 RNA

The inactivated sample was transferred to the upper reservoir of the spin column in a 2.0ml collection tube and was spun for 60 second at 13000 rpm. The flow through in the collection tube was poured off and the collection tube assembled back to the spin column. The Viral RNA was extracted by adding 500ul of prepared wash solution 1 (AW1) into the spin column with collection tube and centrifuged at 13,000rpm for 60 seconds. The flow through in the collection tube was poured off and the collection tube assembled back to the spin column. Again, 500ul of prepared wash solution 2 (AW2) was added in to the spin column with collection tube it was centrifuged at 13,000rpm for 60 seconds. The flow through in the collection tube was poured off and the collection tube assembled back to the spin column. It was spin dried by centrifuging at 13,000 rpm for 60 second to remove residual ethanol. The collection tube was discarded and replaced with 1.5ml micro-centrifuge tube. The Viral RNA was eluted by adding 60ul of Elution Buffer in to the spin column in the 1.5ml micro-centrifuge tube and centrifuged at 13,000 rpm for 60 seconds. The spin column was discarded and the purified RNA in the 1.5ml micro-centrifuge tube was obtained. The process was accompanied with Negative Extraction control (NEC) to monitor the process of

extraction. The process was carried out in a biosafety cabinet: After RNA Extraction personal protective equipment and all materials used was sanitized, autoclaved and disposed.

2.6.3. Amplification

This procedure involves converting the extracted viral RNA into complementary DNA (cDNA) through reverse transcription, followed by amplification of these specific DNA sequences (Enitan, 2020). Quantitatively this method was utilized to identify the Envelope gene (E), Nucleocapsid Gene (N), and Open Reading Frame (ORF) gene within the genomic region of the virus This was achieved using SARS-COV-2 PCR Liferiver Kit manufactured by Shanghai ZJ Bio-Tech Co., Ltd. The rRT-PCR test was conducted with R-Biopharm's Rida cyclor (Zrcycler, Version 1.0, 2017-12-14).

The Cycle Threshold (Ct) values obtained from the samples were analysed within specified ranges, serving as an indicator of viral load. Additionally, positive controls (viral RNA plasmid) and negative controls (sterile Nuclease-free water) were included in the process to ensure accuracy (Hur et al., 2020). A specimen was classified as positive for SARS-CoV-2 if the Ct values of two or three target genes fell below the cutoff (<40) and if the internal control showed amplification. Conversely, a specimen was deemed negative if all Ct values were above the cutoff or if there was no amplification observed. Any result falling outside these criteria or without amplification was interpreted as inconclusive.

2.7. Serology Analysis using Rapid antigen diagnostic test kits (RDTs)

Test was performed using four different types of rapid diagnostic antigen test kits (RDTs) Standard Q (RDT-1), Abbott (RDT-2), Biobase (RDT-3) and Diapro (RDT-4) in accordance with the manufacturer's instructions.

2.7.1. Test Principle

The Rapid Antigen Diagnostic testing kit is a qualitative immunochromatographic test designed for the swift detection of SARS-CoV-2 nucleocapsid protein antigen (Ag) in oropharyngeal and nasopharyngeal swab samples of human. A membrane strip that has been pre-coated with immobilized anti-SARS-CoV-2 antibody on the test line and mouse monoclonal anti-chicken IgY on the control line forms part of this apparatus.

During the examination, two distinct forms of couplings, specifically human immunoglobulin G (IgG) directed towards SARS-CoV-2 antigen bound to gold (which attaches to the nucleocapsid protein), and chicken immunoglobulin Y (IgY) bound to gold, ascend on the chromatographic membrane. These couplings interact with antibodies against SARS-CoV-2 and pre-applied mouse monoclonal antibodies against chicken IgY, respectively. In the event of a positive outcome, the human IgG specific to SARS-CoV-2 antigen bound to gold and the anti-SARS-CoV-2 antibody will amalgamate to produce a discernible line in the test result section.

The presence of a visible control line is necessary to confirm the validity of the test result.

2.7.2. RDT test procedure

Before initiating the testing process, it's essential to allow all elements of the kit to stabilize at a temperature ranging between 15-30°C for 30 minutes. Subsequently, the test device, enclosed within a foil pouch, is removed and positioned on a sanitized, flat surface. Each test cartridge is identified with a unique sample number, corresponding to the specimen under examination.

The swab's tip is then immersed in the buffer solution within the extraction tube, ensuring sufficient contact with the tube's inner surface through swirling motions repeated at least four to five times. Following swirling, the swab is severed at the designated breakpoint inside the extraction tube, and the cap is securely closed, followed by thorough mixing of the contents.

The nozzle cap of the tube is taken off, and 2-3 drops of the extracted specimen are carefully dispensed vertically into the designated specimen well (S) on the apparatus. Subsequently, it was allowed to undergo an incubation period at ambient temperature for 15-30 minutes, adhering to the manufacturer's instructions (Mak et al.,2020). The intensity of the test line is evaluated in accordance with the manufacturer's guidelines.

Upon completion of the testing procedure, the nozzle cap is sealed, and the used cartridges are disposed following Local regulations and protocols for managing biohazardous waste.

2.7.3. Interpretation of Results

A negative result is indicated when only the control line (C) is present within the result window, and no test line (T) appears. This signifies that the specimen tested did not contain detectable levels of the targeted antigen.

Conversely, a positive result is indicated by the presence of both the test line (T) and the control line (C) within the result window. This confirms the presence of the targeted antigen in the specimen.

An invalid result occurs when there is an absence of both the control line (C) and the test line (T) within the result window after the designated incubation period of the cartridges. Additionally, if only the test line appears without the presence of the control line, the test is also considered invalid (Leixner et al., 2021). This indicates a potential issue with the test procedure or components, and the result cannot be interpreted reliably.

2.8. Data Analysis

The data collected was organized and compiled into an Excel spreadsheet. Graphs and charts were then created using the data within the Excel sheet.

3.0 RESULT

Table 1 shows the demographic presentation of the participants used in this study. Age distribution of participants revealed that 348 (9.4%) were between age group 20-30 years old, 1463 (39.5%) were between age 31-40years old, 1396 (37.7%) were between age group 41-50 years old, 450 (12.1%) were between age group 51-60 and then 61-70 years were 48 (1.3%) which recorded the least population. This study recruited female 376 (10.1%) and males 3329 (89.9%). Based on marital status, single participants were 863(23.3%), married 2830(76.4) and widowed 12 (0.3%). Participants with symptomatic which include fever, weakness, body pains, headache, loss of taste, loss of smell, difficulty in breathing 143 (3.9%) and the asymptomatic participants 3562 (96.1%).

Table 1: Demographic presentation of Participants with Rt-PCR and RDTs

Variables		No tested	% tested	+Ve RtPCR	-Ve Rt PCR	STANDARD			
						Q	ABBOT	BIOBASE	DIA.PRO
Stages of life	20 - 30 years	348	9.4	33	315	10	15	8	6
	31 - 40 years	1463	39.5	91	1372	56	67	36	15

	41 - 50 years	1396	37.7	53	1343	39	47	18	9
	51 - 60 years	450	12.1	18	432	12	16	8	5
	61 - 70 years	48	1.3	6	42	3	6	2	1
Gender	Male	3329	89.9	173	3156	3	6	2	1
	Female	376	10.1	28	348	16	34	20	9
Marital Status	Single	863	23.3	100	763	48	61	19	14
	Married	2830	76.4	100	2730	71	89	52	22
	Widowed	12	0.3	1	11	1	1	1	
Symptoms	Symptomatic	143	3.9	132	59	95	122	52	21
	Asymptomatic	3562	96.1	69	3445	25	29	20	15
Total		3705	100	201	3504	120	151	72	36

In this diagnostic study, data demonstrated accuracy of four rapid antigen tests: Standard Q (RDT-1), Abbott (RDT-2), Biobase (RDT-3) and Dia.Pro (RDT-4) for identifying COVID-19 in comparison using Amplification Cycle threshold value of the gold standard of diagnosis Rt-PCR (Figure 2).

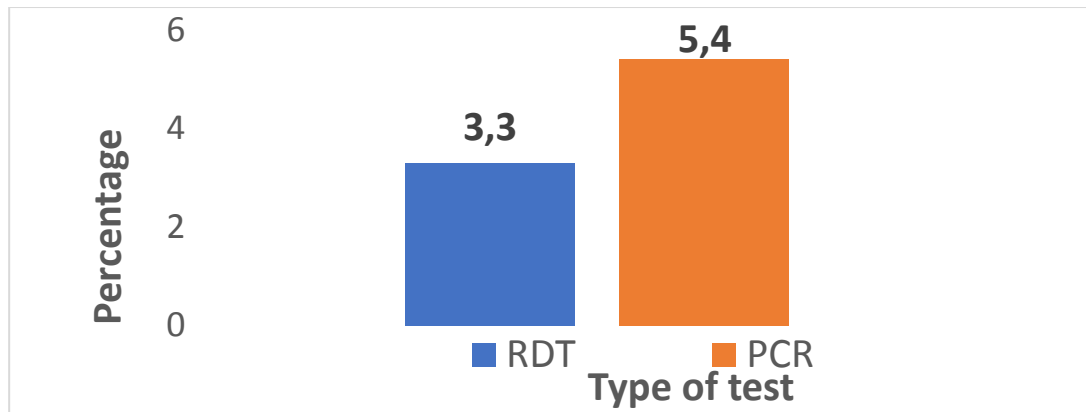


Figure 2: Comparative Prevalence of COVID-19 using RDTs and PCR

Out of the 3705 nasopharyngeal and oropharyngeal swab samples collected and diagnosed for SARS-CoV-2 using RT-PCR, 201 samples tested positive for two to three genes, meeting the cutoff value of <40 cycle threshold as per the kit's guidelines. Samples with cycle threshold (Ct) values less than 40 were deemed positive for SARS-CoV-2. This resulted in a prevalence rate of 5.4% among the total population size (Figure 3).

With RDTs, Standard Q (RDT-1) gave total positive of 120/3705 (3.2%), Abbott (RDT-2) 151/3705 (4.1%), Biobase (RDT-3) 72/3705 (1.9%) and DiaPro (RDT-4) 36/3705 (1.0%). Giving a total prevalence of 3.3% of the total population size (Figure 4).

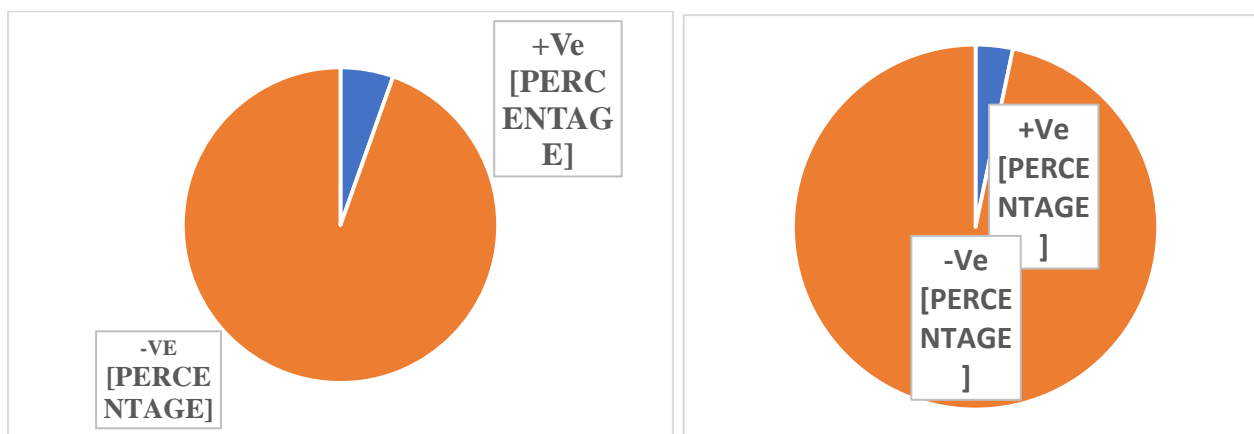


Figure 2: COVID-19 Seroprevalence (Rt-PCR) Figure 3: COVID-19 Seroprevalence (RDT)

The four rapid antigen diagnostic test kits in comparison with the Ct value of the genes of the benchmark of identification of the novel Coronavirus SARS-COV-2 Rt-PCR showed that at Ct value ≤ 30 , number of test that showed positive for Standard Q are ORF (95), N-gene (104) and E-gene (106), Abbott, ORF (110), N-gene (125) and E-gene (129), Biobase: ORF (68), N-gene (70) and E-gene (70) and Dia Pro: ORF (36), N-gene (36) and E-gene (36). At Ct value of the genes $CT > 30$ number of test that showed positive for Standard Q are ORF (25), N-gene (16) and E-gene (14), Abbott, ORF (41), N-gene (26) and E-gene (22), Biobase: ORF (4), N-gene (2) and E-gene (2) and Dia.Pro: ORF (0), N-gene (0) and E-gene (0). Number of negative results obtained for the genes at Ct value of ≤ 30 for Standard Q is ORF (20), N-gene (27) and E-gene (35), Abbott ORF (5), N-gene (6) and E-gene (12), Biobase: ORF (47), N-gene (61) and E-gene (71) and Dia.Pro: ORF (79), N-gene (95) and E-gene (105) as indicated in Table 2.

Number of negative results obtained for the genes at Ct value of ≤ 30 for Standard Q is ORF (20), N-gene (27) and E-gene (35), Abbott ORF (5), N-gene (6) and E-gene (12), Biobase: ORF (47), N-gene (61) and E-gene (71) and Dia.Pro: ORF (79), N-gene (96) and E-gene (105). Number of negative results obtained for the genes at Ct value of > 30 for Standard Q is ORF (61), N-gene (54) and E-gene (46), Abbott ORF (45), N-gene (44) and E-gene (38), Biobase: ORF (82), N-gene (68) and E-gene (58) and Dia.Pro: ORF (86), N-gene (70) and E-gene (60) as indicated in Table 2.

Table 2: Incidence of positive and negative RDT test results in relation to Ct values for ORF, N-Gene and E-Gene of Rt-PCR

		STANDARD Q		ABBORT		BIOBASE		DIA PRO	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
ORF	CT ≤ 30	95	20	110	5	68	47	36	79
	CT > 30	25	61	41	45	4	82	0	86
N-GENE	CT ≤ 30	104	27	125	6	70	61	36	95
	CT > 30	16	54	26	44	2	68	0	70
E-GENE	CT ≤ 30	106	35	129	12	70	71	36	105
	CT > 30	14	46	22	38	2	58	0	60
Total		120	81	151	50	72	129	36	165

Abbott (RDT-2) was seen to have the highest sensitivity of 75.1%, 98.6% NPV and 100.0% specificity and PPV followed by Standard Q which showed 59.7% sensitivity, 97.7% NPV and 100.0% specificity and PPV. Biobase showed 35.8 % sensitivity, 96.4% NPV and 100% specificity and PPV. Dia pro showed the least sensitivity of 17.9 %, 95.5% NPV and 100.0% specificity and PPV.

Table 3: The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the RDT kits.

	TP	FN	FP	TN	Sensitivity	Specificity	PPV	NPV
STANDARD Q	120	81	0	3504	59.7	100.0	100.0	97.7
ABBORT	151	50	0	3504	75.1	100.0	100.0	98.6
BIOBASE	72	129	0	3504	35.8	100.0	100.0	96.4
DIA PRO	36	165	0	3504	17.9	100.0	100.0	95.5

4. DISCUSSION

The study population's median age falls within the 31-40year range, indicating that a significant portion of the participants were middle-aged, with a decline in numbers observed as age increased. Notably, a substantial proportion of participants were between the ages of 31 and 40 (39.5%), while the smallest representation was found among those aged 61-70 years (1.3%). This demographic trend aligns with findings from Malani et al. (2020) and Monod et al. (2021), who observed sustained transmission of SARS-CoV-2 among adults aged 20 to 34 and 35 to 49. Additionally, this age group, spanning from 20 to 49 years, exhibits a lower mortality rate but a higher transmission rate.

Furthermore, the study revealed a predominantly male population, comprising 89.9% of participants, while females represented only 10.1%. This observation is corroborated by research conducted by Gebhard et al. (2020), Jin et al. (2020), and Finelli et al. (2021), all of whom found that males are more susceptible to SARS-CoV-2 infection. Lifestyle factors such as reduced personal hygiene, handshaking, smoking, drinking, and communal living contribute to males' higher risk of contracting the virus (Ciarambino et al., 2021). Conversely, women are more likely to adhere to protective health measures such as mask-wearing, hand hygiene, and social distancing, which are crucial for slowing transmission. Additionally, women generally possess greater knowledge about COVID-19 compared to men. Interestingly, a significant number of males perceive the pandemic as a hoax (Bailey et al., 2020).

These gender disparities in response to COVID-19 align with broader patterns of health behaviour, as discussed by Griffith et al. (2020), emphasizing the importance of public health interventions tailored to men's health needs. Moreover, Abate et al. (2020) highlighted males' increased susceptibility to COVID-19 infection compared to females. Male migration patterns, often driven by economic factors, contribute to higher exposure to the virus. Evidence from the study area suggests that many men defied government-imposed lockdown orders to attend to their businesses, often neglecting mask-wearing and hand hygiene measures, which can lead to the gathering of crowds and increased transmission risk.

Many of the participants were married (76%) while (23.3%) were single and few were widowed (0.3%). This revealed that married and single individuals are more at risk in contacting the disease than the widowed. The married have more access to social activities trying to make ends meet in that case moves around communicate and interact with many people which gives more room to

contracting and spreading the virus. The singles mostly between ages 20-30 also exhibit youthful nature of hang out, parting and clubbing. Most widowed are within ages 51-60- or 61-70-years old stays more indoors than the married and the single.

Participants in the research were sorted into groups depending on whether they exhibited symptoms associated with SARS-CoV-2 infection, such as fever, changes in taste or smell perception, gastrointestinal issues like diarrhoea, respiratory symptoms like cough and shortness of breath, nasal congestion, feelings of tiredness, and muscle or joint pain. Out of the total participants, 143 (3.9%) presented with symptoms, while the majority, 3562 (96.1%), were asymptomatic. Interestingly, both symptomatic and asymptomatic individuals exhibited a high viral load, as indicated by low cycle threshold (Ct) values. This suggests that asymptomatic individuals can harbour a significant viral load without displaying symptoms, making them capable of transmitting the infection similarly to symptomatic individuals.

Both RT-PCR and rapid diagnostic tests (RDTs) demonstrated higher sensitivity in detecting infections among individuals exhibiting symptoms compared to those who did not show symptoms. Since the majority of participants in the study were without symptoms, the lower sensitivity rates reported here compared to a hospital-based study in Italy conducted by Parvu et al. (2021) could be attributed to this distinction. This finding may help explain the high rate of transmission observed in Rivers State, as asymptomatic individuals can unknowingly spread the virus.

RDT-2 showed the highest positive performance of 4.0%, followed by RDT (3.2%), then RDT-3 (1.9%) and the least were seen in RDT-4 (0.9%). RDT-3 showed highest negative performance of 98.1%, RDT-4 had 97.8%, RDT-1 had 96.6% and then RDT-2 had 95.9%. This result is in agreement with those reported by Pray et al. (2021).

RT-PCR yielded a higher prevalence rate of 5.4% compared to RDT at 3.3%. While RT-PCR is less likely to mistakenly classify individuals as having COVID-19 (low risk of false positives), it may miss true cases, leading to false negatives, as the virus RNA can persist in the human system even after recovery for up to a week, potentially resulting in positive RT-PCR results (Moran et al., 2020).

The researchers compared the cycle threshold (Ct) values of the specific SARS-CoV-2 genes in RT-PCR between samples that yielded positive and negative results on the rapid diagnostic test (RDT). They found that Ct values played a pivotal role in determining the outcomes of the RDT. More precisely, samples that tested positive on the RDT exhibited notably lower Ct values for the target SARS-CoV-2 genes compared to those that tested negative. This inverse relationship between Ct values and viral load implies that higher Ct values correspond to lower levels of viral presence. This observation aligns with previous research indicating that sensitivity decreases as Ct values rise, as demonstrated by studies conducted by Porte et al. (2020) and Krüttgen et al. (2021).

In direct contrast with RT-PCR, all RDTs exhibited a higher number of positive results for Ct values below 30 and a lower number of positive results for Ct values above 30. Conversely, a greater number of negatives were observed for Ct values above 30. This indicates that RDTs are more sensitive at detecting high viral loads and recent infections when viral load is very high with low Ct values. Therefore, they serve as valuable tools for frontline testing and rapid response in situations where quick RT-PCR testing is not feasible.

For a patient to be discharged from isolation, the Ct values of the target genes must be above 40, and the RDT must show a negative result. Individuals with lower Ct values, indicative of higher

viral loads, are likely to be more infectious (Singanayagam et al., 2020). This underscores the importance of using both RT-PCR and RDT in tandem to accurately diagnose and manage COVID-19 cases.

Results obtained revealed that RDT-2 (Abbott) have 75.1% sensitivity and 100.0% specificity followed by Standard Q which showed 59.7% sensitivity and 100.0% specificity, Biobase 35.8% sensitivity and 100.0% specificity and lastly Dia pro 17.9 % sensitivity and 100.0% specificity. Similar finding was reported by Kim et al. (2022) for Standard Q (RDT-1) based on their analysis of the 65 positive samples, the specificity and sensitivity of the Standard Q COVID-19 Ag test were 96.0%, and 89.2% respectively. Abdul-Mumin et al. (2021) in their study Field evaluation of specificity and sensitivity of a standard SARS-CoV-2 antigen rapid diagnostic test: A prospective study at a teaching hospital in Northern Ghana demonstrated a sensitivity of 64% and specificity of 81%. An alternative study in Uganda using Standard Q-RDT in the hospital patients and controls revealed a sensitivity of 70.0% (Nalumansi et al., 2021).

The outcome from RDT-2 (Abbott) closely mirrors findings from various studies conducted in different regions, including Ethiopia (74.2%) (Ashagre et al., 2022), Valencia, Spain (79.6%) (Albert et al., 2021), Madrid, Spain (73.3%) (Linares et al., 2020), Switzerland (81.0%) (Ngo et al., 2021), Brazil (84.0%) (Faíco-Filho et al., 2022), Geneva University Hospital (85.5%) (Berger et al., 2021), Saint Maarten, Dutch Caribbean (84%) (King et al., 2021), and Vienna (73.33%) (Wölfl-Duchek et al., 2022). Furthermore, Ashagre et al. (2022) provided insight into the diagnostic performance of the Panbio™ Abbott SARS-CoV-2 rapid kit in a study involving 120 COVID-19 suspected patients at the all-African Leprosy Research and Training (ALERT) Hospital in Ethiopia. They reported the specificity and sensitivity of the Abbott RDT as 100% and 74.2% respectively.

Biobase exhibits sensitivity of 69.3% and specificity of 99.3%, as per information available on www.biobase.com. On the other hand, the Diapro Rapid Antigen kit demonstrated specificity of 100.0% and sensitivity of 17.9%. These findings are consistent with studies conducted by Rivera-Olivero et al. (2022) on the diagnostic performance of seven commercial COVID-19 serology tests in South America. Similarly, Jeewandara et al. (2022) reported comparable results in their research for validation, which involved the comparison of two rapid antigen kits, the Abbott rapid Ag assay and the SD-Biosensor rapid Ag assay with 4786 participants. The overall sensitivity of the SD-Biosensor RAT was 36.2%, while that of the Abbott RAT was 52.6%. Moreover, the Abbott RDT demonstrated higher specificity (99.6%) compared to the SD-Biosensor RAT (97.6%).

To meet the standards recommended by the World Health Organization (WHO) for COVID-19 testing, Rapid Antigen Diagnostic Test Kits should ideally possess a minimum sensitivity and specificity of 80% and 97%, respectively, or greater (WHO, 2021; Peeling et al., 2021).

The results of this study endorse the utility of Rapid Diagnostic Tests (RDTs), particularly the Abbott RDT, as a valuable screening tool for suspected COVID-19 cases, given its close adherence to the WHO's recommended standards. These tests offer rapid results, are simple to administer, transport, and store, and do not necessitate specialized training or skilled laboratory personnel. Consequently, they can be effectively utilized for prompt screening of patients within healthcare facilities in Nigeria. Early identification and isolation of cases are pivotal in slowing transmission rates and ensuring timely clinical intervention for infected individuals, thereby safeguarding the effective functioning of healthcare systems (WHO, 2020, 2021). According to WHO assessments of SARS-CoV-2 Antigen tests, the Abbott RDT emerged as a frontrunner in terms of performance.

In this study, the PPV of all the RDTs shows 100%. That is all are able to give a positive result in the actually disease condition. The Negative predictive value which shows negative (normal) test as

actual or truly free of disease are shown among the RDTs as follows; RDT 1(97.7%), RDT2 (98.6%), RDT3 (96.4) and RDT4 (95.5%). RDT 2 shows the highest NPV and the least was seen with RDT4. Similar findings were reported by Jeewandara et al. (2022) comparing Abbott and Standard Q gave PPV and the NPV for Abbott RDT (92.1% and 95.7%) Standard Q RDT as (77% and 87.1%). The Negative Predictive value was significantly better with the Abbott RDT.

5. CONCLUSION

In instances where rapid rRT-PCR testing isn't feasible, Rapid Diagnostic Tests (RDTs) have demonstrated strong performance, particularly in patients with low Ct values, making them a crucial frontline diagnostic tool. Despite having lower sensitivity compared to rRT-PCR, rapid antigen diagnostic tests exhibit high specificity and positive predictive value (PPV), effectively detecting both symptomatic and asymptomatic SARS-CoV infections. Their rapid results, cost-effectiveness, and minimal resource requirements enable early detection, facilitate mass testing, and assist in isolating infected individuals to reduce domestic and societal spread rates. Although rRT-PCR remains the gold standard due to its superior sensitivity and specificity, rapid antigen tests offer an affordable and practical solution, especially in middle and low-income countries like Nigeria, with specificity reaching 100% and sensitivity values exceeding 75% for SARS-CoV-2 detection. Confirmatory rRT-PCR testing should be considered following negative antigen test results in symptomatic individuals and positive antigen test results in asymptomatic individuals (CDC, 2020). This study highlights the varying seroprevalence, sensitivities, and specificities of different RDTs in use, with Abbott (RDT2) demonstrating the best sensitivity, specificity, PPV, and NPV. Abbott kit is among the rapid antigen diagnostic test kits recommended by the Nigeria Centre for Disease Control (NCDC).

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