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Diagnostic Precision of Immunoglobulin (IgM/IgG) and Rapid Antigen Tests in the Detection of COVID-19 in Port Harcourt, Rivers State

Moore-Igwe, Beatrice. W[1], Kemzi Nosike Elechi-Amadi[1], Mike-Ogburia, Moore Ikechi[1], Gilbert Grace Ezra[1], Ben-Chioma, A. E.[1]

[1]Department of Medical Laboratory Science, Faculty of Science, Rivers State University, Nkpolu-Oroworukwo, P.M.B. 5080, Port-Harcourt, Rivers State, Nigeria.

Corresponding Author: Beatrice Wobiarueri Moore-Igwe

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ABSTRACT

An urgent diagnostic challenge is presented by the COVID-19 pandemic, particularly in resource-limiting settings. Numerous rapid antigen and antibody (IgM/IgG) tests are frequently used to diagnose COVID-19, but it is still unclear how accurate these tests are. With Real-Time Polymerase Chain Reaction (RT-PCR) serving as the gold standard approach for the detection of SARS-CoV-2. This comparative study was conducted to assess the diagnostic accuracy of the COVID-VIRO® Rapid antigen and COVID-PRESTO® rapid antibody (IgG/IgM) test kits. Nasopharyngeal swabs and capillary blood samples were obtained from 200 subjects suspected of being exposed to COVID-19. RT-PCR was used to detect the presence of SARS-CoV-2 in the nasopharyngeal swab specimens using the Magnetic Induction Cycler platform (MIC), the nasopharyngeal swabs were also assayed for the viral proteins using COVID-VIRO® rapid antigen test kits. IgM/IgG antibodies were detected in capillary blood samples using COVID-PRESTO® Rapid antibody test kits. The MIC qPCR cycler nCov assay's sensitivity and specificity were compared to those of the rapid test kits. Out of 200 samples, RT-PCR detected 90 as positive and 110 as negative. The sensitivity and specificity of the rapid antigen test were 95.5% and 100%, respectively. IgM rapid antibody test sensitivity and specificity were 38.9% and 90%, respectively, while IgG antibody test sensitivity and specificity were 40.7% and 82.6%, respectively. The findings from the investigation indicate that the rapid antigen test kit is more suitable than the rapid antibody (IgG/IgM) test kits since it performed better and had a stronger association with the gold standard RT-PCR. It is also more sensitive, specific, and accurate.

KEYWORDS: Coronavirus Disease 2019 (Covid-19), Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2), Polymerase Chain Reaction (PCR), Deoxyribonucleic Acid (DNA), Immunoglobulin (Ig), and Cycle Threshold (Ct)

1. INTRODUCTION

The outbreak of unexplained severe pneumonia in Wuhan, Hubei Province of China, in late December 2019 led to the discovery of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel member of the beta coronavirus family that causes the highly contagious respiratory disease coronavirus disease 2019 (COVID-19) (CDC, 2022). Global public health has remained threatened by the COVID-19 since its outbreak. Since the first reported case of this infection, the virus has spread globally, bringing with it a devastating impact on many

nations; it has stretched healthcare systems of most countries. By January 31, 2022, the virus had spread to 223 nations, with over 378 million confirmed cases and more than 5.6 million fatalities, creating a pandemic of unprecedented proportions in the 21st century. As long as the epidemic persists, the health of the entire world's population is seriously threatened, hence it is anticipated that these numbers will keep rising (WHO, 2022).

Nigeria reported its first COVID-19 case, which was brought into the country from Italy on February 27, 2020, and was identified by the World Health Organization (WHO) as one of the 13 African nations with the highest risk of COVID-19 outbreaks, due to the country's failing healthcare system (Marbot, 2020). As at February 2022, there were 253,340 confirmed COVID-19 cases in Nigeria, with 3,136 deaths (NCDC 2022). Given the reality of the virus and the need for consistent differential diagnosis and epidemiological surveillance, it is expedient to have readily available, simple-to-use, highly sensitive and specific diagnostic tests to appropriately identify cases and mitigate the spread of COVID-19 in the country.

The majority of SARS-CoV-2 diagnostic tests fall into two categories: (1) molecular tests that use isothermal nucleic acid amplification and real-time reverse transcription polymerase chain reaction (RT-PCR) to detect SARS-CoV-2 RNA in respiratory samples; and (2) COVID-19 Rapid diagnostic immunological tests that either detect SARS-CoV-2 antigens in respiratory samples or anti-SARS-CoV-2 antibodies (primarily IgG and/or IgM) in plasma, serum or whole blood (Veyrenche *et al.*, 2021).

The current gold standard for the diagnosis of COVID-19 is the real-time RT-PCR (RT-PCR) test for detecting viral nucleic acid in upper and lower respiratory tract specimens (Veyrenche *et al.*, 2021). Even though RT-PCR testing is one of the most precise, sensitive, and reliable laboratory methods for detecting SARS-CoV-2 infection, it is only possible to run a certain number of assays daily particularly given the economic realities in a country like Nigeria. Other practical drawbacks of this detection method include the requirement for expertise as well as expensive, specialized equipment and laboratories, the comparatively long time needed to provide results extended turnaround time, and the potential for false-negative results when virus loads are low in clinical specimens. Due to these drawbacks, RT-PCR is not ideal for rapid and simple patient testing, rapid diagnostic test (RDT) kits using the specific antigen for SARS-CoV-2, are available for the detection of specific antibodies and proteins associated with the virus (Paradiso *et al.*, 2020)

Since RDTs are widely available and simple to use, they may increase the effectiveness of SARS-CoV2 testing. However, little is known about the diagnostic precision of immunoassays such as anti-SARS-CoV-2 IgG/IgM and antigen-based rapid diagnostic tests (RDTs). The purpose of this study is to comparatively assess the diagnostic performance of commercially available rapid SARS-CoV-2 antigenic test (COVID-VIRO®) and rapid immunological test (COVID-PRESTO®) to the Gold Standard RT-PCR test for the identification of COVID-19 infections in Port Harcourt.

2. MATERIALS AND METHODS

2.1 Study Design

A simple random sampling method was used for this study. This study involved 200 test subjects, from which nasopharyngeal and capillary whole blood samples were collected and used for the diagnostic molecular and immunologic (antigen and antibody) assays listed below:

1. Magnetic Induction Cycler (MIC) qPCR cycler version 2.10. The Magnetic Induction Cycler (MIC), is a Real-time polymerase chain reaction (RT-PCR) equipment a 48-well

rotary-based small qPCR device, that uses magnetic induction to heat things and force airflow to cool things down. The device includes two or four detection channels with excitation and emission spectra that cover the most popular qPCR dye.

II. The COVID-VIRO® quick test cassette for the Covid-19 antigenic test

III. Covid-19 serological and antibody (IgM and IgG) test employing a quick test cassette (COVID-PRESTO®).

2.2 Study Area

This comparative study was conducted in Port Harcourt, the capital of Rivers State, Nigeria, with a population of almost 2 million. Port Harcourt is a commercial city in Nigeria's South-South geopolitical zone, popularly known as the Niger Delta. This study involved suspected COVID-19 adult subjects (male and female), between the ages of 19 and 67 years, of which 123 suspected cases, 13 overseas travelers, 17 contact tracing cases, and 47 work-based examinations. The average age for males (145) and female (55) subjects were 45±5.0 and 42±3.0 respectively.

2.3 Data Collection

Following proper disclosure of the study's significance to the individuals and acquisition of their informed consent, the following demographic data was gathered: the test's purpose, the subjects' name, age, and gender,

2.4 Sample Collection

The collection of nasopharyngeal swab samples from patients was carried out by qualified Medical Laboratory Scientists, while they were outfitted in the necessary personal safety gear (PPE). The nasopharynx was probed with a synthetic fibre-tipped swab with a thin plastic shaft until resistance was felt there. Before being removed, it was rotated three times at 180 degrees while being in situ for up to 10 seconds. Following the swabbing process, the swab applicator was disconnected, and each absorbent swab was put into a vial containing 3 mL of viral transport media (VTM), which is composed of Hanks' balanced salt, 0.4% fetal bovine serum, HEPES, antibiotics, and antifungal agents.

Vials were delivered to the laboratory right away utilizing a triple packaging technique, accompanied by a cold chain, before testing at 4°C (from the point of sample collection to the analyzing laboratory. The nasopharyngeal specimens were kept between 2 and 8 degrees Celsius for up to 72 hours when a delay in results was anticipated. The side of the fingertip was pricked with a sterile lancet to collect whole blood samples (capillary blood specimens), and a big drop of suspended blood was allowed to develop. A 10µl auto-filling capillary micropipette was used to collect this blood.

The blood sample was then released by applying pressure to the micropipette's bulb and depositing it in the proper well of the test cassette. Retests were only conducted on the same patient when the initial test result was negative. All samples were processed in a facility with full personal protective equipment and biosafety level-2 enhancement (BSL-2 +).

The following formulas were used to determine the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy: Sensitivity = (TP/TP+FN) ×100, Specificity = (TN/TN+FP)×100, PPV = (TP/TP+FP)×100, NPV = (TN/TN+FN)×100, and Accuracy = (TN+TP/TP+FP+FN)×100. Where TP= True Positive, FN=False Negative, TN= True Negative, and FP = False Positive.

2.5 STATISTICAL ANALYSIS

The general data regarding the patients were described using descriptive statistics. A mean and standard deviation were used to depict continuous data. Numbers and percentages were used to present categorical data, with a 95% confidence level deemed statistically significant. The confidence intervals were

computed using Schoonjan's F Med Calc statistics program.

3. RESULTS

Table 3.1 indicate the findings of the Real-time Polymerase Chain Reaction (RT-PCR), rapid antigenic test, and rapid antibody (IgM and IgG) tests, table 3.2 shows the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy obtained while table 3.3 shows the performance traits of the four patients whose COVID-VIRO® fast antigen test results were negative but who tested positive for RT-PCR.

Table 3.1: Comparison of Results from the Different Techniques

	PCR	Antigen	IgM	IgG
Positive	90	85	46	56
Negative	110	115	154	144

Table 3.2: Performance Characteristics of the Methods/Techniques

	Antigen (95% CI)	IgM (95% CI)	IgG (95% CI)
Sensitivity	95.5 (88.9-98.8)	38.9 (28.8-49.7)	40.7 (30.5-51.5)
Specificity	100 (96.7-100)	90.0 (82.8-94.9)	82.6 (74.1-89.1)
PPV	100 (96.7-100)	76.1(63.2-85.5)	65.6 (54.2-75.5)
NPV	96.5 (91.3-98.6)	64.3(60.2-68.2)	63.0 (58.4-67.3)
Accuracy	98.0 (94.9-99.4)	67.0 (60.1-73.5)	63.7 (56.6-70.4)

Table 3.3: Characteristics of the Patients with False Negative COVID-VIRO® Results

Patient	Age	Sex	COVID-VIRO® Results	Ct values of E, N, and Orf1 Genes respectively
1	21	M	Negative	32.61, 31.56, 33.15
2	35	M	Negative	31.35, 29.47, 31.13
3	37	F	Negative	34.19, 28.31, 34.43
4	45	M	Negative	35.21, 32.33, 33.96

DISCUSSION

This study assessed the diagnostic accuracy of two rapid diagnostic tests, (a) COVID-VIRO®, which is intended to identify SARS-CoV-2 antigens from nasopharyngeal swab samples, and (b) COVID-PRESTO®, which is intended to identify SARS-CoV-2 antibodies (IgG and IgM) from capillary whole blood samples within 15 minutes. The results of these diagnostic assays were compared to the results of the industry-recognized RT-PCR assay (MIC qPCR thermocycler version 2.8) for the identification of SARS-CoV-2 infection.

Findings drawn from this study indicated that 85 of the 90 RT-PCR-positive samples were accurately detected by the COVID-VIRO® antigen rapid diagnostic test, yielding a very high sensitivity of 95.51% and specificity of 100% with an accuracy of 98% and no false positive result seen. The findings of this study are consistent with those of earlier research on the diagnostic performance of COVID-VIRO® RADT by Courtellemont *et al.* (2021) which revealed (a sensitivity of 96.7% and a specificity of 100%). The study by Cassuto *et al.* (2021) also corroborated with the observations in the present study as their comparative assessment of the COVID-VIRO® RADT yielded a sensitivity of 96.8% and 100% specificity. The WHO-recommended requirements of ≥80% sensitivity and ≥97% specificity was met in the current study.

Even when Cycle threshold (Ct) values were >32 as determined by the MIC qPCR thermocycler (Bio-molecular systems) assay, the rapid antigen detection test (RADT) continued to work with strong diagnostic performance. Four out of the RT-PCR-positive samples had COVID-VIRO® RADT results that were negative, and the Ct values for these four patients were all higher than 32. This is in contrast with the research work of Mak *et al.* (2020) and Krüttgen *et al.* (2021), which showed that RADTs perform better in samples with low RT-PCR Ct values.

Due to the false safety impression on the patients as they entered a highly contagious phase, the false negatives as seen in this study may result in some SARS-COV-2 infected individuals going undetected during the incubation period, which could have dangerous consequences (Fernandez-Montero *et al.*, 2021). The majority of the patients in this study with low (Ct 25-30) to moderate (Ct 30-35) Ct values (corresponding to high S (RADTs). This, however, contrasts somewhat with that of Kruttgen *et al.* (2021), which demonstrated varying sensitivities in accordance with Ct values of viral load.

Samples with medium (Ct 5-30), low (Ct 30-35), and extremely low (Ct >35) virus loads, respectively, had sensitivities of 95%, 44.8%, and 22.2%. The results in this study have shown that the COVID-VIRO® RADT could be just as sensitive as the RT-qPCR in identifying SARS-COV-2 infected individuals in low resource settings (awaiting RT-PCR results in a testing hub where testing is done at no cost). This is in agreement with the work of Cassuto *et al.* (2021).

The COVID-VIRO® RADT in this investigation also had very high negative and positive predictive values (96.5% and 100%, respectively), which slightly contradicts the findings of Cassuto *et al.* (2021), whose negative and positive predictive values were also very high (99.5% and 100%, respectively). Although not significantly, negative predictive value (NPV) decreases with decreased prevalence, and while this is important to know, low NPV can be disastrous for patients with negative RADT tests because it allows them to freely interact with other people while they are unaware that they are virus carriers (Fernandez-Monteroab *et al.*, 2021).

The findings of this study also demonstrated that the COVID-PRESTO® rapid antibody (IgM/IgG) test kits, which utilized capillary whole blood samples, had low overall sensitivity for both IgM and IgG antibodies (38.9% and 40.7%, respectively), and specificities of 90% and 82.6%, respectively, for both IgM and IgG. The diagnostic performance of such quick strip immunoassays was assessed in several recent studies using venous blood samples. While their specificity for IgM antibodies was 100%, which did not match ours, the sensitivity found in this study was comparable to that reported by (Nicol *et al.*, 2020), who recorded a sensitivity of 43.8% for IgM for samples collected after 7 days of symptom onset; the overall specificity was comparable to that reported by (Li *et al.*, 2020), who recorded a specificity of 90.63% in their prospective study on the diagnostic performance of rapid antibody testing.

The results, however, were very different, with (Chapentier *et al.*, 2021) reporting sensitivities of 67% and 97.1% for IgM and IgG antibodies, respectively, and specificities of 100% (for IgM) and > 96% for both IgM and IgG antibodies. However, no information regarding the time each patient had had symptoms was gathered in this study at the time blood samples was collected. According to earlier research, the immune system produces IgM and IgG antibodies between 6 and 21 days after contracting SARS-COV-2 (Wölfel *et al.*, 2020). The IgM and IgG antibodies to SARS-COV-2 only become detectable 1-3 weeks after symptoms first appear, according to the COVID-PRESTO® fast antibody test's manufacturer. The intensity and temporal dynamics of the humoral response to SARS-CoV-2 are still unknown. It is widely acknowledged that IgM is the initial antibody in the immune response to viral infections, acting as the first line of defense before the formation of adaptive, high-affinity IgG responses, which give more potent long-term immunity (Prazuck *et al.*, 2020).

The low performance of the COVID-PRESTO® RDT used in this study may have been brought on by a high percentage of false negatives during the early stages of infection, which is directly related to the low antibody

titers in the first few days following COVID-19 infection. In a study by Wölfel *et al.* (2020), it was discovered that 4 days after infection, both IgM and IgG titers were low or undetectable. Additionally, it was found that the prevalence of antibodies was lower than 40% in patients within a week of the onset of symptoms and quickly rose to 94.3% (IgM) and 79.8% (IgG) from day 15 following the commencement (Zhao *et al.*, 2020).

The COVID-PRESTO®'s diagnosis accuracy in this investigation was lower than in other prospective studies (74.5% and 72.1% for IgM and IgG antibodies, respectively), but this finding was predicted given the study subjects' varied geographic locations and genetic make-ups. The WHO's requirements for a screening test was met by this particular COVID-19 antigenic fast detection test (sensitivity 80%, specificity 97%). The COVID-PRESTO® antibody-based quick diagnostic tests, on the other hand, frequently return false negative results shortly after the onset of symptoms.

CONCLUSION

The results of this study demonstrate that COVID-VIRO® rapid antigen tests (AAZ, Boulogne Billancourt, France) have high diagnostic accuracy even when used independently during the early stages of the disease because they are highly positive in individuals with low to moderate Ct values, which correspond to high viral loads (RT-PCR). Antibody testing shouldn't be utilized to determine a person's SARS-CoV-2 infection status. Antibody testing is not currently advised to evaluate SARS-CoV-2 immunity following COVID-19 vaccination or to determine whether an individual needs to get vaccinated.

Declaration by Authors

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