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Research Article

SARS-CoV-2 Infection Screening Using Two Serological Testing Methods

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Summary: The challenges associated with adequate deployment of nucleic acid amplification tests (NAATs) in developing countries underscores the important role of simple but sensitive and specific serological testing kits in COVID-19 diagnosis. Presently, there are a number of point-of-care tests for Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) screening. However, the reliability of these test kits is poorly documented and hence, needs to be ascertained. This study was therefore designed to determine the sensitivity and specificity of two serological test kits for COVID-19 screening with the view to providing necessary information on the suitability of their deployment as routine test kits for SARS-CoV-2 in Nigeria. Forty-seven (47) asymptomatic adults who had been tested for SARS-CoV-2 with the real-time reverse-transcriptase polymerase-chain reaction (RT-PCR) were enrolled into this study. Blood samples were obtained for qualitative determination of serum IgM and IgG antibodies to the S-antigen of SARS-CoV-2 using a commercially available IgM and IgG Rapid Diagnostic Test (RDT) and enzyme linked immunosorbent assay (ELISA). The association between the test kits (ELISA and RDT) and PCR in diagnosing COVID-19 was determined using the Fisher's Exact test at $P<0.05$. The sensitivity and specificity of the test kits were determined using ROC while the Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR), Diagnostic Odds Ratio (DOR) and accuracy were calculated as appropriate. Twenty-eight (59.6%) of the study participants had positive PCR result. ELISA and RDT identified 20 (42.6%) and 13 (27.7%) participants respectively as having anti- SARS COV-2 specific antibodies. ELISA had a better sensitivity performance, NPV, PLR, DOR and accuracy than the RDT while the RDT had a better specificity performance than ELISA. The proportion of participants with anti-SARS-CoV-2 IgM antibody identified using ELISA was significantly higher compared with RDT. In contrast, the proportion of participants with positive anti- SARS COV-2 IgG antibody identified using RDT was significantly higher compared with ELISA. ELISA has a better sensitivity for detecting anti-SARS-CoV-2 Spike-protein specific antibodies than the RDT. However, combination of RDT and ELISA for the detection of anti-SARS-COV-2 antibodies might be useful for population COVID-19 screening

Keywords*:* Anti-SARS-CoV-2 antibody, COVID-19 screening, ELISA, Rapid diagnostic strip, Serology

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) is a pandemic which continues to highlight the need for different types of validated diagnostics tests with a view to meeting global demands (Weissleder *et al*., 2020). This demand is more pronounced in developing countries where availability and accessibility to molecular testing could be limited.

To provide epidemiologic data and to contain outbreaks, a number of diagnostic testing methods and kits have been developed for COVID-19 with each method performing distinct roles. These include the nucleic acid, serological, antigen, and ancillary tests (FDA 2020; Weissleder *et al*., 2020; Yang *et al*., 2020) The nucleic acid amplification tests (NAATs) are the most widely used and recommended test for the diagnosis of SARS-CoV-2, identification of strains or mutations and triage of patients. NATs detect the presence of the viral genome and indicate an active, current infection but cannot detect a prior infection including recently resolved ones (Jacofsky *et al*., 2020).

Another important testing method is the serological test. This test detects the anti-SARS-CoV-2 antibodies (mainly IgG, IgM and IgA) produced by the immune system. This test is not indicated for early diagnosis and screening for active early infection of COVID-19 as there is a time lag (6 – 7 days) for immune response to the antigenic viral invasion

culminating in SARS-CoV-2 specific antibody production. However, essential information on the immune status, prior infection and the status of a given population related to their ability to contract or resist infection is based on the antibody status (Jacofsky *et al*., 2020).

The challenges associated with adequate deployment of NAATs in developing countries including Nigeria underscores the important role of cost-effective, simple but sensitive and specific serological testing kits in COVID-19 diagnosis. This will facilitate reduction in the pressure on NATs and hasten clinical decision making. In addition, the fund saved from the procurement of NAATs equipment can be channelled towards procuring these relatively cheap serological test kits with better turn-around-time.

Presently, there are a number of point-of-care tests for SARS-CoV-2 diagnosis however, the reliability of these test kits is poorly documented and hence, needs to be ascertained. This study was therefore, designed to determine the sensitivity and specificity of two serological test kits for COVID-19 diagnosis with the view to providing necessary information on the suitability of their deployment as routine test kits for SARS-CoV-2 in Nigeria..

MATERIALS AND METHODS

Study Population: Forty seven (47) SARS-CoV-2-asymptomatic adults were enrolled into this study from The Infectious Diseases Centre, Olodo, Ibadan, Oyo State, Nigeria. The real-time reverse-transcriptase polymerase-chain reaction (RT-PCR) assay was used to confirm the status of all the study participants using nasal and pharyngeal swab specimens following WHO guideline (WHO 2020b)

Sample collection: Five millilitres (5 ml) of venous blood was obtained from each study participant and was dispensed into plain sample bottles to obtain sera as appropriate. Participants with positive result for PCR assay were followed up till discharge and 5 ml of venous blood was also obtained at the point of discharge. An aliquot of each serum was used for the RDT while the remaining portion of the serum was stored at -20^{0}C for ELISA analysis.

Ethical consideration: The study was approved by the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee (UI/EC/20/0233) and informed consent was obtained from each study participant.

Laboratory analyses: Qualitative determination of IgM and IgG antibodies to the Spike-protein of SARS-CoV-2 was done using two methods (ELISA and Rapid Diagnostic Test) following the manufacturers' instruction. Briefly, the ELISA kits qualitatively detect human IgM and IgG antibodies specific for SARS-C.

The specific antibodies in the sample bind with the SARS-CoV-2 spike-protein antigen in pre-coated wells of microtitre plates. Thereafter, horseradish peroxidase (HRP) conjugated mouse anti human IgG and IgM respectively is added to the wells to form antigen-antibody-HRP conjugated secondary antibody complex, which is amplified using chromogen/substrate. Absorbance at 450 nm is measured and compared with the kits stipulated cut-off values to determine if a sample contains SARS-CoV-2 spike-protein specific IgM or IgG. Samples with absorbance value equal to or above the cut-off values are regarded as positive for anti-SARS-CoV-2 spike-protein specific IgM or IgG antibodies while samples with absorbance value less than the cut-off are regarded as negative for SARS-CoV-2 spike-protein specific IgM or IgG antibodies. For RDT, the sample reacts with SARS-CoV-2 S-antigen coated on the conjugate pad. If specific antibodies are present in the sample, the anti- SARS-CoV-2 IgM and/or IgG antibody (of the S-antigen - anti-SARS-CoV-2 IgM/IgG antibody) binds to mouse anti-human monoclonal antibodies on the IgM and IgG lines. The presence of anti-SARS-CoV-2 IgM and/or IgG is indicated by a visible test line on the IgM and IgG lines in addition to the visible control line.

Estimation of diagnostic performances*:* The sensitivity and specificity of the test kits were determined using ROC. Thereafter, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR), Diagnostic Odds Ratio (DOR) and accuracy were calculated as earlier described (Molinaro 2015; Trevethan 2017).

Statistical Analysis

Statistical analysis was carried out using the SPSS statistical software version 23.0 for windows. The association between the test kits (ELISA and RDT) and PCR in diagnosing COVID-19 was determined using the Fisher's Exact test. *P*-value less than 0.05 was considered as statistically significant.

RESULTS

The presence of SARS-CoV-2 based on the 3 methods is presented in Table 1. Twenty-eight (59.6%) of the study participants had positive PCR result. ELISA and RDT identified 20 (42.6%) and 13 (27.7%) participants respectively as having anti- SARS-CoV-2 specific antibodies (Table 1).

In order to understand the specific class of antibody detected by the ELISA and RDT, the results observed in Table 1 were stratified. As shown in Table 2, the proportion of participants with anti-SARS-CoV-2 IgM antibody identified using ELISA was significantly higher compared with RDT. In contrast, the proportion of participants with positive anti-SARS-CoV-2 IgG antibody identified using RDT was significantly higher compared with ELISA. ELISA showed that all the 47 participants had no anti-SARS-CoV-2 IgG antibody as at the point of sample collection but the RDT showed that 25.5% of the study participants had anti-SARS-CoV-2 IgG IgG antibody at diagnosis.

Table 1:
Diagnosis of COVID-19 using three different methods

	PCR		ELISA		RDT	
	Yes	No	Yes	No	Yes	No
At enrolment (n = 47)	28	19	20	27	13	34

Table 2:
Proportion of study participants with positive and negative SARS-CoV-2 IgM and IgG antibodies using ELISA and RDT

Antibodies	**ELISA**	**RDT**	χ^2	***P*-value**
IgM				
Positive	20 (42.6%)	1 (2.1%)	22.136	0.000*
Negative	27 (57.4%)	46 (97.9%)		
IgG				
Positive	0 (0.0%)	12 (25.5%)	13.75	0.000*
Negative	47 (100.0%)	35 (74.5%)		

*Significant at $P<0.05$

Table 3:
Summary of comparative diagnostic performances of ELISA and RDT in participants screened for SARS-CoV-2 infection

Indices of Diagnostic Performance	**IgM ELISA**	**IgM RDT**	**IgG RDT**
Sensitivity	64.3%	3.6%	42.9%
Specificity	89.5%	100.0%	100.0%
Positive Predictive Value	90.0%	100.0%	100.0%
Negative Predictive Value	63.0%	41.3%	54.3%
Positive Likelihood Ratio	6.12	0.036	0.0
Negative Likelihood Ratio	0.40	0.964	0.571
Diagnostic Odds Ratio	15.3	0.037	0.0
Accuracy	0.74	0.426	0.7

Comparative diagnostic performances of ELISA and RDT in participants screened for SARS-CoV-2 infection are shown in Table 3. ELISA had a better sensitivity performance, NPV, PLR, DOR and accuracy than the RDT while the RDT had a better specificity performance than the ELISA. The RDT had 100% specificity as it had negative reaction for all the 17 study participants with negative PCR results. Of these 17 participants however, 2 had positive ELISA reaction hence; the specificity of ELISA was lower (89.5%) compared with the RDT.

Dynamics of IgM and IgG changes at enrolment and at discharge are shown in Table 4. The ELISA result alone was considered as no participant was found to have IgG at enrolment. It was observed that 15 participants who had anti- SARS-CoV-2 IgM antibody at diagnosis still sustained it till discharge, 5 participants who had anti-SARS-CoV-2 IgG antibody had it at discharge, 5 participants have SARS-CoV-2 IgM at diagnosis and at discharge, 3 participants with anti-SARS-CoV-2 IgM antibody at diagnosis did not have at discharge while 6 participants had anti-SARS-CoV-2 IgM antibody at diagnosis and anti-SARS-CoV-2 IgG antibody at discharge.

Table 4:
Dynamics of changes in IgM and IgG at enrolment and at discharge (using ELISA)

	Pattern of result	**Number of patients**
IgM		
At diagnosis	At discharge	
+ve	+ve	15
-ve	+ve	5
+ve	-ve	3
-ve	-ve	5
IgG		
At diagnosis -ve	At discharge +ve	6

+ve = Positive, -ve = Negative

DISCUSSION

Viral RNA detection using nucleic acid amplification test (NAAT) such as RT-PCR is the recommended test for SARS-CoV-2 infection diagnosis (La Marca *et al*., 2020). However, the long turn-around-time (TAT), owing to the numerous logistics involved in NAAT (Weissleder *et al*., 2020) as well as the poor infrastructure and resources in developing countries clearly underscores the relevance of rapid serological testing kits, which are used for binary qualitative

results determination, in revealing the factual epidemiology of COVID-19.

Relative to PCR, ELISA and RDT accurately detected 71.4% and 46.4% respectively of the study participants with SARS-CoV-2 infection. This observation indicates that the SARS-CoV-2 detection capacity of ELISA is more than that of RDT and could therefore be deployed in poor resource settings even in asymptomatic individuals. Generally, it is believed that the accuracy of serological tests improves (can be near 100%) when samples are obtained 20 days after infection when the immune response would have fully evolved (Weissleder *et al*., 2020).

SARS-CoV-2 is one of the seven coronaviruses known to have infected humans hence; issues of cross-reactivity in diagnostic tests cannot be overemphasized. In fact, false-negative results using RT-PCR due to variability in viral load and sampling have been reported in ~30% of COVID-19 patients and are thus of great concern in NAAT (Ai *et al*., 2020; Tang *et al*., 2020; Yang *et al*., 2020). Therefore, evaluation of serological testing kits performance, is critical in ensuring true identification, solely from among people who are known to have SARS-CoV-2 infection, all those who do indeed have SARS-CoV-2 infection (i.e. true positives), and avoiding categorization of other people as not having SARS-CoV-2 infection when in fact they do have it (i.e. false negatives) (Pereira 2016; Trevethan 2017). In this study, ELISA had a better sensitivity performance, NPV, PLR, DOR and accuracy than the RDT. This observation supports the earlier observed higher proportion of COVID-19 patients detected by ELISA compared with the RDT. In contrast, the RDT had a higher specificity compared with ELISA. The RDT had negative reaction for all the 17 participants with negative PCR results but ELISA had negative reaction for 15 of the 17 participants. The seemingly false-positive result observed in ELISA cannot be substantiated as false-negativity is also a concern in NAAT (Tang *et al*., 2020). Zhao *et al*. (2020) reported that the sensitivity of antibody based test kits, which was lower (38.3% vs 66.7%) at the early phase of SARS-CoV-2 infection overtook that of NAAT and reached over 90% 12 days after onset of illness.

Diagnostic accuracy of antibody tests depends on its purpose and its interpretation varies. Diagnosing SARS-CoV-2 infection in symptomatic patients requires that the test has sensitivity as high as 90% with a slight reduction in specificity acceptable. In contrast, high specificity as high as 98%, is essential when antibody tests are to be used in the determination of when to terminate social isolation (La Marca *et al*., 2020). In this study, 12 out of the 13 participants with positive RDT result had IgG while only one had IgM. This observation highlights possible challenges associated with the use of RDT for SARS-CoV-2 infection screening using IgM antibody. Our observation corroborates the report of Zhang *et al*.,)2020) which showed that some patients tested with RDT were more positive for IgG than IgM at the moment of hospitalization and 5 days later; but had an earlier IgG than IgM seroconversion.

Although RDT has a better specificity compared with ELISA in this study, its poor sensitivity (3.6%) shows that ELISA has a better SARS-CoV-2 infection detection ability than RDT. The RDT even detected IgG in 25.5% of the participants who were found to be IgG-negative using ELISA. This observed discrepancy could suggest cross-reaction of the S-protein on RDT with other antigens, probably other human coronaviruses, aside SARS-CoV-2 S-protein. This suggestion could be alluded to by the observed higher sensitivity of ELISA compared with the RDT. However, the observed high specificity might suggest that RDT could be useful in making clinical decision on imunnoprotection status and when to return to normal activities following social isolation (La Marca *et al*., 2020) as all the participants with negative PCR result were equally detected as SARS-CoV-2 S-antigen specific IgM and IgG free. Due to the observed better sensitivity of ELISA over RDT and the better specificity of RDT over ELISA, it could be suggested that a combination of RDT and ELISA for SARS-COV-2 antibodies might be useful for population screening. It was suggested in the reports of Zhao *et al*. (2020) and Özçürümez *et al*. (2020) that a combination of molecular and serological tests could facilitate accurate diagnosis of patients with COVID-19 at different stages of the disease.

Diagnostic accuracy of serological testing kits is dependent on the evolvement of immune response to the immunogen of interest (La Marca *et al*., 2020). Generally, in response to infections or immunization, IgM antibodies are elicited first but disappear after a few weeks. However, IgG antibodies are produced at the same time or 2 to 3 days later and titres usually remain high for months or years (Racine and Winslow 2009; WHO 2020a).

The immune response dictates the seroconversion time and varies in COVID-19 patients. Zhao *et al*. (2020) reported median seroconversion rates of 93.1%, 82.7% and 64.7% for total antibody, IgM and IgG at 11, 12 and 14 days respectively in COVID-19 patients. Wölfel *et al*., 2020) reported that 50% of their studied patients had seroconversion after 7 days while all the patients seroconverted after 14 days. Similarly, IgG seroconversion was observed in 285 COVID-19 patients within 19 days of onset of symptoms (Long *et al*., 2020). In this study, it was observed that the pattern of IgM and IgG antibody response varies and this variation determines the outcome of serological testing. All (20) the participants with positive IgM antibody result had negative IgG antibody result when

tested using ELISA. This observation is in line with the asymptomatic status of the enrolled participants. The report of Liu *et al*., 2020) showed that anti-SARS-CoV-2 S-specific IgM and IgG antibodies were not detectable from days 0 to 3. However, specific IgM antibodies became detectable at day 4 and peaked at about day 20 before gradual decline which became marked 4 weeks after onset of symptoms. They also showed that specific IgG antibodies were detectable from day 7 and peaked at about day 25 and remained high in concentration even, after 4 weeks of infection. It has been reported that the sensitivities of antibodies for SARS-CoV-2 specific antigens increase with the increasing number of days of illness. Zhao *et al*. (2020) showed that patients in the latter phase (days 15 – 39 post infection) had 100.0%, 94.3% and 79.8% sensitivities for total antibody, IgM and IgG respectively as against 45.5% sensitivity of NAAT. The pattern of changes of antibodies observed in this study further substantiate the dynamics involved in the interpretation of anti-SARS-CoV-2 antibody test results as individual humoral immune responses against SARS-CoV-2 infection may slightly differ. Usually, positive antibody result to SARS-CoV-2 antigen(s) indicates past infection with SARS-CoV-2 which can be recent infection (concurrence of IgM and IgG) or an infection of more than a few weeks ago (IgG only). In contrast, negative antibody result to SARS-CoV-2 connotes no infection, recent infection in the last 14 days or infection with antibody levels below the level of detection of the immunoassay (WHO 2020a). The technicalities involved in the interpretation of antibody detection kits due to the dynamics of individual immune responses to infections further situates NAAT as the cornerstone diagnostic assay for SARS-CoV-2 infection. Furthermore, Wang *et al*. (2020) reported that there exist a small proportion of patients with difficulty in rapidly gaining immunity against SARS-CoV-2 even up to 50 days after their symptoms onset. It is apparent therefore, that care needs to be taken when using anti-SARS-CoV-2 antibodies for COVID-19 clinical diagnosis and determination of discharge criteria.
Small sample size was a limitation in this study.

It could be concluded from this study that ELISA has a better sensitivity for the detection of anti-SARS-COV-2 S-antigen specific antibodies than the RDT. However, combination of RDT and ELISA for the detection of anti-SARS-COV-2 antibodies might be useful for population COVID-19 screening. A large population study is still required to confirm the observations in this study before anti-SARS-CoV-2 S-antigen specific antibodies ELISA can be deployed as routine test kits for SARS-CoV-2 diagnosis and monitoring in Nigeria.

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