

# Evaluation of SARS-CoV-2 Antibody Rapid Diagnostic Test kits (RDTs) Innovita® and Real Time-Polymerase Chain Reaction (Rt-PCR) for COVID-19 Infection Surveillance in Kaduna State, Nigeria.

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## ABSTRACT

**Background:** The Real Time Polymerase Chain Reaction (Rt-PCR) is the gold standard diagnostic test for SARS-CoV-2, however there are several invalidated antibody-based tests available for community screening. This study compared the performance of the SARS-CoV-2 antibody test kits and Rt-PCR in the diagnosis and surveillance of COVID-19 infection. **Methodology:** The study was conducted at the Infectious diseases control center (IDCC), Kaduna and Hamdala Motel isolation center, of Kaduna State, using a cross-sectional study design. The sample size, 521 was achieved by convenience sampling method over a period of three months. Venous blood samples, collected from consenting clients and patients were analyzed for SARS-CoV-2, using the kit by Innovita® Biological Technology CO., LTD China. Oral/nasopharyngeal swabs were collected and analyzed using the Rt-PCR. Data analysis was carried out using the SPSS version 23. Proportions were compared using Chi square tests for proportions. Level of statistical significance was set at  $p \leq 0.05$ . **Result:** Our study revealed low sensitivity for both IgM (14.4%) and IgG (15.6%), using the RDT antibody kits, specificity of 92.9% and 98.6% for IgM and IgG. Using PCR-positive cases as true positives, the accuracy of the test was 67.8% and 72.0% for IgM and IgG, respectively. The positive and negative predictive values for IgM were 48.9% and 69.7%. For IgG, the corresponding values were 83.9% and 71.2%. **Conclusion:** The SARS-CoV-2 Innovita® RDT kit is far from ideal as a tool for the rapid sero-prevalence survey of Covid-19 with its low sensitivity. More efforts are urgently required to access standard diagnostic kits, with high sensitivity/specificity index when compared with the gold standard, for community screening, especially at the regions not easily accessible due to a number of reasons.

**Keywords:** Rapid Diagnostic Test kits, Real Time-Polymerase Chain Reaction (Rt-PCR), SARS-CoV-2



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## INTRODUCTION

The outbreak of the new coronavirus (SARS-CoV-2) infection has spread to every continent of the globe, thus, forcing us to adapt to living with it. Since the emergence of this virus, there has been a lot of challenges in trying to control it, this led to a gradual halt in almost all economic and social activities both locally and internationally.<sup>[1]</sup>

The SARS-CoV-2 virus is highly contagious and spreads easily within populations. Factors that aid the spread of the virus include; population density, environment, social activities, culture.<sup>[2]</sup> The clinical presentations of COVID-19 range from asymptomatic or mild symptoms to severe illness leading to multiple organ failure and death. Mortality from the disease is associated with advanced age, immune-compromised states, and those with comorbidities.<sup>[3]</sup>

In response to the rising COVID-19 pandemic, several diagnostic test manufacturers have developed rapid and easy to use test kits to facilitate testing both within and outside the laboratory. Among which are test kits for the detection of antibodies in blood samples. However, the WHO recommends the validation of these test kits in appropriate populations and settings.<sup>[4]</sup> This is to prevent false positive or negative categorization of people which may hinder disease control efforts.

The availability of a locally validated and adapted antibody test kit will improve the ease and access to a screening tool for point of care testing in primary, secondary and tertiary health care centers thereby, strengthening the local testing capacity for COVID-19 in resource constrained settings. The detection of asymptomatic infections using a locally validated serological test kit will also improve community surveillance capacity, which will aid in decision making on measures to control the viral spread such as when to enforce quarantine or isolation.

The ability to detect the immune response using a rapid and reliable serology test has a huge advantage in the assessment of the viral spread in

the population and the level of herd immunity. The comparative advantage of utilization of the validated test kits for COVID-19 screening in the general community cannot be overemphasized. This will enable the identification of previously undiagnosed asymptomatic infected persons who might have recovered and developed antibodies and may serve as a reservoir for convalescence plasma to be utilized for those with severe infection as a form of passive immunity, in the absence of specific drug therapies or vaccines. The screening can also help measure and determine how the society gradually eases back to normalcy and the number of patients coming for voluntary testing as well as reduction in stigma, which are in accordance with the targets of Goal 3 of the Sustainable Development Goals (SDG). This pilot study therefore, sought to assess and compare the performance of the SARS-CoV-2 antibody test and Real Time Polymerase Chain Reaction (Rt-PCR) in the diagnosis of COVID-19.<sup>[5]</sup>

## METHODOLOGY

### Study Design

The study was conducted at isolation centers and in the four local government areas within the metropolis using a cross-sectional study design. We employed a design as described by Momeni *et al* to assess the accuracy and precision of a candidate SARS-CoV-2 test kit, an antibody-based rapid diagnostic kit for COVID-19.<sup>[6]</sup>

### Study area/population

Kaduna State is located at the Northern part of Nigeria's High Plains, with a population of approximately 7.7 million. The State is bordered by seven States. The State was ranked number four by total area of land and number three by population in Nigeria.<sup>[7]</sup>

All clients, who presented for community screening (at designated specimen collection centers at Kaduna North, Kaduna South, Chikun,

Igabi local government areas) and those confirmed positive for SARS-CoV-2 virus by RT PCR at the State isolation centers (Infectious disease center, Kakuri and Hamdalla Hotel) who gave consent for the study.

### Sample size

Five hundred and twenty-one participants were enrolled by convenience sampling technique and specimens were collected over a period of three months across the different sites of collection from all consenting clients.

### Inclusion criteria

All those who presented for community screening and confirmed cases at the isolation centers for SARS-CoV-2 infection.

### Exclusion Criteria

All neonates and infants were excluded from the study.

### Methods

For the purpose of this evaluation, the diagnostic test kit by Innovita® Biological Technology CO., LTD China, was used on venous whole blood samples (4.0mls) dispensed into Ethylene diamine tetra-acetic acid (EDTA) anticoagulated bottles. According to the manufacturer of the antibody test kits, the specificity had been evaluated and was found to be 100% (95% CI:94.20% to 100%) for both IgM and IgG, while the sensitivity evaluated on COVID-19 cases was 87.3% (95% CI: 80.40% to 92.0%) for both IgM and IgG.

Oral and nasopharyngeal swabs of the same participant were analyzed at the Genomics laboratory of the Barau Dikko Teaching Hospital, Kaduna State, using the real time Polymerase chain reaction technique for nucleic acid detection and quantification, following RNA extraction (with Life River extraction kits, Shanghai, China) using primers obtained from Genefinders Company LTD, South Korea. For the evaluation, samples from COVID-19 cases were obtained during disease condition and previously

confirmed by PCR, were used as the true positives. Assuming a confidence (accuracy) of 0.95%, reliability (precision) of 0.90% and a sample size of 521, true positive value, true negative value and positive and negative predictive value of the antibody was assessed using the formula:

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN}$$

$$\text{Acceptability criterion} = \text{accuracy of } \geq 95\%$$

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100$$

$$\text{Specificity} = \frac{TN}{TN + FP} \times 100$$

$$\text{Positive Predictive Value} = \frac{TP}{TP + FP} \times 100$$

$$\text{Negative Predictive Value} = \frac{TN}{TN + FN} \times 100$$

### Data Analysis

Data analysis was carried out using the Statistical Package for the Social Sciences (IBM SPSS) version 23. Results were presented as charts, tables, and figures as appropriate. Proportions were compared using Chi square tests for proportions. Level of statistical significance was set at  $p \leq 0.05$ .

### Ethical consideration

Ethical approval from Kaduna State Ministry of Health was obtained. Written, informed consent was obtained from participants and the privacy and confidentiality of all data was ensured during the study. The study was at no cost to the participants and they were free to withdraw at any point without loss of rights or privileges.

### Data Management

All data was safely secured using alphanumeric pass-worded computers with up-to date commercial antivirus software. Each entry was backed immediately using a secure external hard drive. Hard copies were kept in a separate, safe office under lock. Only authorized individuals had access to the data.

## RESULTS

**Table 1: Sociodemographic characteristics of participants n= 521**

Characteristics	Cases Frequency (%)	Healthy Frequency (%)
<b>Age (years)</b>		
<30	71 (42.5)	138 (39.0)
30-49	66 (39.5)	135 (38.1)
>49	30 (18.0)	81 (22.9)
<b>Sex</b>		
Male	101 (60.5)	193 (54.5)
Female	66 (39.5)	161 (45.5)

Serum samples were tested from 521 persons 167 of whom were COVID-19 cases and 354 were apparently healthy individuals. The mean age of the participants was 35.2± 15 years. Majority of the cases (42.5%) and healthy (39.0%) participants were less than 30 years of age. There were more male cases (60.5%) and male healthy participants (60.5%) than females.

### IgM and IgG response in negative control samples and PCR-confirmed COVID-19 patients

Seven percent (7%; n= 25/354) of sera from healthy blood donors tested positive for IgM in the assay, while only about one percent (n= 5/354) tested positive for IgG. (Tables 2 and 3).

### IgM and IgG response in PCR-confirmed COVID-19 patients

Altogether, 24 of 166 (14.5%) samples from PCR confirmed COVID-19 patients tested IgM positive

and 26 (15.7%) tested IgG positive (Tables 2 and 3).

### Assay sensitivity, specificity and accuracy

Based on the results described above and summarized in Tables 2 and 3, the assay showed a sensitivity of 14.4% (n= 24/167) and 15.6% (n= 26/167) for IgM and IgG, respectively. The assay showed an overall specificity of 92.9% (n= 329/354, 25 false positive) and 98.6% (n= 349/354, 5 false positive) for IgM and IgG respectively. Using PCR-positive cases as true positives, the accuracy of the test was 67.8% (n= 353/521) and 72.0% (n= 375/521) for IgM and IgG, respectively. The positive and negative predictive values (the likelihood of being a case given a positive test result, and the likelihood of being healthy given a negative test result) for IgM were 48.9% (n= 24/49) and 69.7% (n= 329/472), respectively. For IgG, the corresponding values were 83.9% (n= 26/ 31) and 71.2% (n= 349/490).

Table 2. Comparisons of IgM results for 167 PCR-positive COVID-19 cases and 354 healthy individuals

	Cases Frequency (%)	Healthy Frequency (%)	Total Frequency (%)
IgM positive	24 (49.0)	25 (51.0)	49 (100)
IgM negative	143 (30.3)	329 (69.7)	472 (100)
<b>Total</b>	<b>167</b>	<b>354</b>	<b>521</b>

Chi square ( $\chi^2$ ) = 7.11 (2-18) p value = 0.008

There were statistically significant differences found between IgM status and PCR status of cases

Table 3. Comparisons of IgG results for 167 PCR-positive COVID-19 cases and 354 healthy individuals

	Cases Frequency (%)	Healthy Frequency (%)	Total Frequency (%)
IgG positive	26 (83.9)	5 (16.1)	31 (100)
IgG negative	141 (28.8)	349 (71.2)	490 (100)
<b>Total</b>	<b>167</b>	<b>354</b>	<b>521</b>

Chi square ( $\chi^2$ ) = 40.63 (CI 5-100) p value = .00001

There were statistically significant differences found between IgG status and PCR status of cases

### As sociation between sociodemographic characteristics and IgM status

Table 4. Association between sociodemographic characteristics and IgM status

Characteristics	IgM Status		P value
	Positive Frequency (%)	Negative Frequency (%)	
<b>Age (years)</b>			0.957
<30	20 (40.8)	138 (40.0)	
30-49	18 (36.7)	135 (38.8)	
>49	11 (22.4)	81 (21.2%)	
<b>Sex</b>			0.001*
Male	39(79.6)	255 (54.0)	
Female	10(20.4)	217 (46.0)	

\*Statistical Significance

There was a statistically significant difference found between sociodemographic characteristics (P=0.001) and IgM status of cases.

**Association between sociodemographic characteristics and IgG status**  
**Table 5. Association between sociodemographic characteristics and IgG status**

Characteristics	IgG Status		P value
	Positive Frequency (%)	Negative Frequency (%)	
<b>Age (years)</b>			0.138
<30	17 (54.8)	192 (39.2)	
30-49	11 (35.5)	190 (38.8)	
>49	11 (9.7)	108 (22.0%)	
<b>Sex</b>			0.015*
Male	24(77.4)	270 (55.1)	
Female	7(22.6)	220 (44.9)	

There was a statistically significant difference found between sociodemographic characteristics, gender (P=0.015) and IgG status of cases.

#### **Association between sociodemographic characteristics and PCR positivity**

There were no statistically significant differences found between sociodemographic characteristics (Age, P=0.002; Sex, P=0.428) and PCR positivity status of cases.

## **DISCUSSION**

The present study evaluated a commercial rapid test for detection of SARS-CoV-2-specific IgM and IgG. Our results showed an overall very low sensitivity of IgM (14.4%) and IgG (15.6%). A study carried out by Li et al in China in contrast to our study findings reported an overall high testing sensitivity of 88.7% and 90.6% specificity. The specificity results for our study for IgM (92.9%) and IgG (98.6%) were higher than what was reported in the Li *et al* study.<sup>[8]</sup>

Globally, there are some other types of coronaviruses that affect humans that are endemic or even occur as epidemics. These include the 229E, HKU1, NL63 and OC43 strains.<sup>[9]</sup> The picture of significant false positivity observed in this study may possibly be due to the cross reaction from a previous infection with one of the strains of those other viruses mentioned above.

It cannot be said for certain the commonality of the corona viruses as the agents responsible for the flu, but it has been estimated that about 20% of cases of the flu could be as a result of the coronaviruses.<sup>[10]</sup> The specificity for IgG detection of the rapid test

evaluated in this study was similar to what was reported in another study carried out in China, of an enzyme-linked immunosorbent assay (ELISA), which had a specificity 97.5%.<sup>[11]</sup> However, the sensitivity of our study was very low as compared with that of the ELISA which was also 97.5%.

Our study generally showed a poor sensitivity performance of antibody tests for both IgM and IgG. The negative predictive values for the rapid tests in this study were not very high, suggesting that this rapid test may not be useful for detecting past infections and possible immunity. It is not known when infection occurred for individuals in this study.

The timing of the development of SARS-CoV-2-specific antibodies is variable. The humoral response kinetics to SARS-CoV-2 infection have not been fully understood, however it has been shown that reactive IgA, IgM, and IgG antibodies have been detected as soon as 1 day after symptom onset.<sup>[12]</sup> In other previous studies, the antibodies were detected 10 to 15 days after symptom onset. The median time to the development of total antibody, IgM, and IgG has been estimated as 11, 12, and 14 days, respectively.<sup>[13,14]</sup>

A chi-square test of independence was performed to examine the relation between IgM and IgG status and client's Covid-19 status. The relation between these variables was significant,  $X^2 = 7.11$  (CI 2-18),  $p = .008$ , for IgM and Covid-19 status and  $X^2 = 40.63$  (CI 5-100)  $p$  value= .00001 for IgG and Covid-19 status of the participants.

We also investigated the possible associations between IgM, IgG and PCR positivity and sociodemographic characteristics. The associations found were those between IgM and

IgG status and gender. According to the previous studies, gender has not been shown to have significant association with antibodies to SARS-CoV-2. A study conducted in Spain did not show any statistically significant association with presence of IgM and IgG antibodies to SARS-CoV-2 and gender.<sup>[15]</sup>

Another study conducted in the United States of America similarly did not find any association between the presence of IgM and IgG antibodies to SARS-CoV-2 and gender.<sup>[16]</sup> In another study conducted by Zeng *et al* among 331 patients confirmed SARS-CoV-2 infection, it was found that level of IgG antibody in mild, general and recovering patients did not show any difference between males and females.<sup>[17]</sup>

However, the average IgG antibody level in female patients tended to be higher than that of in male patients in severe infections. It was observed that in comparison to male patients, most of the female patients generated a relatively high level of SARS-CoV-2 IgG antibody in severe infection. It was also reported that, the generation of IgG antibody in the females tended to be stronger than the male patients in the early phase of the disease.<sup>[18]</sup> There are inconsistent findings from various studies on the associations between gender and SARS-CoV-2 antibody positivity. It is necessary for additional studies to be carried out to explore further and validate the relationship between gender and SARS-CoV-2 antibody positivity.

### CONCLUSION and Recommendation

The SARS-CoV-2 Innovita® RDT kit is not ideal for the rapid sero-prevalence survey of COVID-19 as we demonstrated low sensitivity with its use. The specificity and sensitivity of the Innovita® test kit for detecting SARS-CoV-2 antibody as claimed by the manufacturer need to be reevaluated for specific populations.

The use of standard rapid kits will ensure the efficiency of large testing strategies, shorten turnaround time as regards results generation,

eliminate the need for numerous highly trained manpower, and does not necessarily need to be performed in a laboratory facility. The RT-PCR, remains the most reliable diagnostic tool for COVID-19 analysis, however, to be an effective alternative to this and have a significant role in the diagnosis/surveillance of COVID-19, RDT kits must have both high sensitivity and specificity.

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