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*Research Paper*

# COVID-19 ANTIBODY TESTING; WHICH WAY NIGERIA?

**Reuben Eifediyi[a,c,d], Cyril Erameh[a,b,c], Oladapo Oladeinde[c,d], Peter Akhideno[a,b,c] and Sylvanus Okogbenin[a,c,d]**

[a]Department of Obstetrics & Gynaecology, Irrua Specialist Teaching Hospital, Irrua, Nigeria.
[b]Department of Internal Medicine, Irrua Specialist Teaching Hospital, Irrua, Nigeria.
[c]Immunology & Vaccinology Unit, Institute of Lassa Fever Research & Control, Irrua Specialist Teaching Hospital, Irrua, Nigeria.
[d]Department of Obstetrics & Gynaecology, Ambrose Alli University, Ekpoma, Nigeria..

**Abstract**

There is no doubt now that COVID-19 poses an unprecedented threat to public health causing a severe crisis in the global economy and resulting in many deaths. So far, it has left many families all over the world in grief, and Nigeria is not an exception. There is a need to boost local, national, and international resilience to Covid-19 and this requires leadership from us all. The first crucial element of the COVID-19 exit strategy is massive testing (for both infection and immunity) so that healthy people can return to work and those who are infected can get appropriate treatment. Presently, Nucleic Acid-Based Testing is used for diagnosis in Nigeria. This is expensive, time-consuming requiring high technical know-how. Nigeria, a mono-economy nation will need to restart her economy, and the present strategy of testing for COVID-19 using molecular-based testing is rather expensive and slow with poor coverage. Hence the adoption of testing protocols and guidelines of the developed world which is largely molecular-based might not work in Nigeria There exists a serologic antibody-based test that could be utilized alongside nucleic acid testing. This article attempts to highlight the process of an immune response, discuss the available testing techniques for COVID-19 including their promise and peril, and then develop an algorithm for cost-effective testing strategy in a manner to increase accessibility.

Key words: SARS-CoV-2, COVID-19, Antibody testing, Antibodies, Algorithm, Validation.

## INTRODUCTION

Since the first case of COVID-19 (caused by the Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) was discovered in Nigeria on 27th February 2020,(Federal Ministry of Health 2020) Nigeria, just like other nations of the world have witnessed a

steady increase in the number of cases and deaths recorded from the disease. As a response to combat the spread of the disease, nations of the world (Nigeria inclusive) instituted lockdowns, downscaling human activities with the potential for significant negative impact on several sectors including the aviation industry, the economy, etc.

The WHO recently advocated testing as a key strategy in returning nations back to normalcy.(WHO 2020a) As the world was unprepared for the pandemic, most nations have struggled to keep pace with the challenges offered concerning testing and treatment of this disease. At the moment, the Reverse Transcriptase Polymerase Chain Reaction Testing (RT-PCR), a molecular test, is the most commonly used test for COVID-19 diagnosis. It is expensive and slow to yield results. Serological testing using antibodies developed by the body in response to COVID-19 is another useful method of testing for the SARS-CoV-2.(Zhao et al. 2020)

Nigeria, one of the worst-hit countries in sub-Saharan Africa with about 25 molecular laboratories to diagnose COVID-19 spread across the nation as of 20$^{th}$ May 2020 had carried out a little less than 40,100 RT- PCR tests.(Nigeria Centre for Disease Control (NCDC) 2020) This translates to testing less than 201 out of a million Nigerians. This relatively low level of testing according to the Nigerian Centre for Disease Control (NCDC) is not unconnected with the huge financial implications of conducting RT PCR tests at such a large scale which the country is not able to bear. To this end and with the explosion of COVID-19 cases in certain regions of the country, the NCDC and the Federal Ministry of Health in a bid to ramp up testing across the nation and have a grip on the spread of the disease is considering population-based antibody testing which is a cheaper and faster form of testing for Covid-19 compared with RT PCR tests.(Zhao et al. 2020)

Most of the patients with COVID-19 show mild to moderate symptoms. Mortality usually occurs as a result of acute respiratory distress syndrome, septic shock, multiple organ failure, bleeding, and coagulation dysfunction. All these are featured at the cellular level by pneumonia, lymphopenia, exhausted lymphocytes, and elevated serum levels of proinflammatory cytokines characterized as a cytokine storm. Therefore, the host immune system is thought to participate strongly in the pathogenesis of COVID-19.

We, therefore, seek to discuss the science of antibody testing, its advantages, drawbacks and make recommendations on its utilization in Nigeria

## Science of immune system response to Covid-19

The human body's immediate response to infection by SARS CoV-2 is described as a non-specific innate response in which white blood cells are recruited to fight against the virus. They slow the progress of the virus and may stop it from developing symptoms. This is followed by an adaptive response in which the body forms antibodies against the virus binding onto them and destroying them. These responses may be strong enough to prevent the individual from developing symptoms of the disease. (WHO 2020b) The antibodies produced are called immunoglobulins (Ig) and Ig G and M are examples. Studies have reported antibody detection as early as three to four days after the patient developed symptoms(Abbasi 2020) with much higher levels detectable by the third and fourth weeks post-onset of symptoms. While IgM levels subsequently begin to decline and reach its nadir by the fifth week and disappearing by the seventh week, IgG levels persist beyond the seventh week.(Sethuraman et al. 2020) It is important to note that the early production of antibodies does not mean the early elimination of the virus. How the COVID-19 virus circulates in the presence of antibodies is an interesting question. It is also known that cellular immunity is generated concomitantly with humoral immunity.(Wang et al. 2020)

## Testing for COVID-19

Several tests have been used to diagnose COVID-19 infections. These could be serologic (use of blood or serum) and non-serologic (use of throat, saliva, nasal, or other body fluids). Serologic antibody tests could be qualitative (lateral flow assays) and quantitative or Enzyme-Linked Immunosorbent Assay (ELISA) based. A substantial number of commercially available COVID-19 antibody test kits are not ELISA based. They are lateral flow assays that give a simple positive or negative result with no quantitative information. They are cheap and easy to use(Zhao et al. 2020) and depending on how they are employed may be useful for disease serosurveillance. The lateral flow assay is also called the Point-of-Care test. Unlike RT PCR tests (also referred to as molecular or nucleic acid-based test), antibody tests are not intended to identify active SARS CoV-2 infections. Instead of detecting viral genetic material in the throat or

nasal swabs, antibody tests reveal markers of immune response- IgM and IgG antibodies that for most people show up in the blood more than a week after they start to show symptoms or when symptoms may already be waning.

## Antibody testing for COVID-19 and therapies

According to Krammer's team and Dutch team, the antibody tests for COVID-19 tests will be critical in the weeks and months ahead when they may be used for disease surveillance, therapeutics, return to work screenings, and more.(Abbasi 2020)

Serological antibody testing could be deployed in several ways: It could be employed in turning antibodies into therapies i.e. screening plasma of recovered patients for antibodies to SARS CoV-2 and transfusing such plasma to critically ill patients. This form of treatment proven to be safe is currently being done on an experimental level is known as convalescent plasma.(Abbasi 2020; Joyner et al. 2020)

Furthermore, the transfusion of convalescent plasma and hyperimmune globulin derived from it to persons who have been exposed to the virus to render passive immunity to them is being considered. A potential unintended consequence of this could also be addressed by antibody testing. COVID-19 survivors who undergo this therapy may not develop immunity thus putting them at risk of re-infection. Antibody testing could be utilised in determining their antibody status post-recovery, identifying those with no immunity or very low immunity who would become candidates for vaccination when a vaccine becomes available.(Abbasi 2020) Furthermore, quantitative antibody tests such as ELISA which determine antibody titres could be useful in determining the degree of protection an individual has based on the amount of antibody possessed with higher antibody titres correlating with higher degrees of protection against COVID-19. A test similar to ELISA is chemiluminescence assays developed by Diazyme laboratories in Poway, California. It measures antibody levels by generating a light signal which is proportional to SAR CoV-2 IgM antibodies.(Abbasi 2020)

## Validation of Covid-19 antibody tests

The validity of a test is its ability to accurately identify diseased or non-diseased subjects.(Maxim et al. 2014) Rapid serological test kits for antibodies against COVID-19 are currently available. However, few of these test kits have been validated and given Emergency Use Authorisation (EUA) by the relevant agencies including the Food and

Drug Administration. Those without EUA must state they have clinically validated their tests using specimens from patients with PCR confirmed infections. (Abbasi 2020)We must understand the limitations of serologic testing recognising that it takes time to mount a detectable immune response and to use them for the right reasons. It could falsely label persons who are positive as negative and persons who are negative as positive. For instance, a false-positive antibody test result may create a false sense of protection against COVID-19 and the individual may change their lifestyle predisposing them to be infected or potentially infecting others posing serious public health challenges. This brings to the fore the critical issue of validation of test kits and its appropriate use

Before the introduction of any COVID-19 antibody test kit in Nigeria, there must be a validation of such test kits. Although the regulatory agencies frown at it, many companies are ready to sell COVID-19 antibody tests without authorisation howbeit with some stipulations.(Abbasi 2020) The Irrua Specialist Teaching Hospital, a centre for research, diagnosis, treatment, and control of Lassa fever and other viral haemorrhagic fevers is planning to collaborate with state governments, partners, and medical institutions to validate these tests and make them available and widely used.

For validation of any test, scientists must understand how diagnostic and screening tests are used to detect the presence or absence of disease in individuals as clinicians rely on these tests to make critical decisions. The validity of any new test especially antibody testing must be assessed by comparing it with the gold standard. (Maxim et al. 2014) The gold standard for diagnosis of COVID-19 is molecular testing (RT PCR) which is expensive, time-consuming, and not readily available.

Table 1 below is a contingency table that forms part of a tool in assessing the validity of a test. It lists the true disease status in the columns and the observed results of the test in the rows. It groups individuals into four test categories highlighting logical possibilities for true disease state and possible test outcome.(Eifediyi, Reuben; Eigbefoh, Joseph; Omorogbe 2015)

Measures of test validity include sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio. The sensitivity of a clinical test refers to the ability of the test to correctly identify patients with the disease. A test with 100%

sensitivity identifies all patients with the disease. The specificity of a clinical test refers to the ability of the test to correctly identify those patients without the disease.

$$\textbf{Sensitivity} = \frac{a}{a+c} = \frac{\text{True positive}}{\text{True positives + False negatives}}$$

$$\textbf{Specificity} = \frac{a}{b+d} = \frac{\text{True negatives}}{\text{True negatives + false positives}}$$

While it is desirable to have a test that is highly sensitive and specific it may not be possible at all times and then the test has to be structured in a manner to trade off some sensitivity for specificity.

The positive predictive value is the percentage of positive tests that are truly positive. While the negative predictive value is the percentage of negative tests that are truly negative. The likelihood ratio is used concerning the utility of the test. It refers to how much more likely is it that a patient who tests positive has the disease compared with the one who tests negative.

$$\textbf{Positive predictive value} = \frac{a}{a+b} = \frac{\text{True positives}}{\text{True positives + False positives}}$$

$$\textbf{Negative predictive value} = \frac{d}{c+d} = \frac{\text{True negatives}}{\text{True negatives + False negatives}}$$

$$\textbf{likelihood ratio} = \frac{\text{Sensitivity}}{1 - \text{Specificity}}$$

**Justification of the Irrua Specialist Teaching Hospital COVID-19 Diagnostic and Case Management Algorithm 2020.**

Nigeria is faced with a PCR test shortfall amidst incredible demand. Its health system may, therefore, consider subbing in serology tests. A false negative serology result in an acutely symptomatic patient who is replicating and shedding viruses has serious public

health consequences. Serologic antibody tests not only can confirm suspected cases after the infection, but they can also reveal who was infected and did not know it. Crucially, antibody testing can also be used to return people with immunity back to the workforce or keep them there beginning with health professionals and emergency first responders. Implementing widespread antibody testing is a critical step towards reopening the system while acknowledging the science of immune response and reports elsewhere. With the current situation in Nigeria, the "algorithm" (see figures 1 and 2 below) was developed to utilize the lateral flow assays and PCR techniques. More so, some COVID-19 patients may not generate specific IgM or IgG antibodies after SARS-CoV-2 infection; thus, testing for SARS-CoV-2 specific antibodies only is not a good standard to detect infection. However, when used in combination with the nucleic acid testing method, there may be an improvement in the accuracy of SARS-CoV-2 detection and cost-effectiveness.

## CONCLUSION

The timely and accurate diagnosis of SARS-CoV-2 infection is the cornerstone of the efforts to provide appropriate treatment for patients, to limit further spread of the virus, and ultimately to eliminate the virus from the society. The performance of RT-PCR in Nigeria depends on many factors such as the sample types, different stages of the infection, the skill of sample collection particularly from the nasopharynx, the quality, availability, cost, and consistency of the PCR assays being used. These problems lead to a noteworthy delay in early diagnosis and follow up management which in turn poses serious challenges in providing timely life supportive care for severely ill patients and preventive quarantine.(Meyer et al. 2014) Serological testing is advantageous with faster turnaround time and less workload. This algorithm developed is done to optimize the PCR techniques and the clinical value of the dynamics of antibodies. Antibodies testing will play a vital role in various settings, like in suspected cases, close contacts of confirmed cases, and even in confirmed cases to know if antibodies have been induced. It will also be of value in searching for an animal host for SARS-CoV-2. Finally, we recommend a serological testing scheme with PCR and outline necessary flow charts to be implemented for the diagnosis and clinical management of patients for better preparedness in the pandemic period.

**REFERENCES**

Abbasi J. The Promise and Peril of Antibody Testing for COVID-19. JAMA - J Am Med Assoc. 2020;2019:2019–21.

Eifediyi, Reuben; Eigbefoh, Joseph; Omorogbe F. Clinical epidemiology and diagnosis of bacterial vaginosis among pregnant women attending clinics in Irrua Specialist Teaching Hospital, Edo State, Nigeria. Spec Pathog Rev J. 2015;1:18–29.

Federal Ministry of Health. The first case of Corona Virus Confirmed in Nigeria [Internet]. Vol. 1. 2020 [cited 2020 May 11]. p. 1–2. Available from: https://tinyurl.com/y946vlpx

Joyner MJ, Wright RS, Fairweather D, Jonathon W, Bruno KA, Klassen SA, et al. Early Safety Indicators of COVID-19 Convalescent Plasma in 5 , 000 Patients [Internet]. 2020. Available from: https://tinyurl.com/y7g22ob2

Maxim LD, Niebo R, Utell MJ. Screening tests: A review with examples. Inhal Toxicol. 2014;26(13):811–28.

Meyer B, Drosten C, Müller MA. Serological assays for emerging coronaviruses: Challenges and pitfalls. Virus Res. 2014;194(January):175–83.

Nigeria Centre for Disease Control (NCDC). Covid-19 Situation Report: Situation Report 59. Niger Cent Dis Control. 2020;205(May):1–4.

Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA [Internet]. 2020;2019:2019–21. Available from: https://tinyurl.com/yd7np5vo

Wang B, Wang L, Kong X, Geng J, Xiao D, Ma C, et al. Long-term Coexistence of SARS-CoV-2 with Antibody Response in COVID-19 Patients. J Med Virol. 2020;(April):22–7.

WHO. Co V I D - 19 Strategy Up Date [Internet]. 2020a. Available from: https://tinyurl.com/y972fwkb

WHO. Immunity Passports in the context of COVID-19 [Internet]. The Lancet Infectious Diseases. 2020b. Available from: https://tinyurl.com/y82arfla

Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis. 2020;