ISSN: 2634 - 8861

Journal of Infectious Diseases & Case Reports

SCIENTIFIC Research and Community

Review Article

Open Access

Molecular Diagnosis of COVID-19 in Nigeria: Current Practices, Challenges and Opportunities

Seyi Samson Enitan[1*], Richard Yomi Akele[2], Chioma Jane Agunsoye[3], Kayode Abraham Olawuyi[4], Amarachi Joy Nwankiti[5], Adeolu Sunday Oluremi[1], Chukwudi Amaechi Ofodile[6], Ayodeji OluSola Olayanju[2], Ovye Engom Godiya Alaba[7] and Comfort Bosede Enitan[8]

[1]Department of Medical Laboratory Science, Babcock University, Ilishan-Remo, Nigeria

[2]Department of Medical Laboratory Science, Afe Babalola, Ado-Ekiti, Nigeria

[3]Department of Medical Laboratory Science, Covenant University Medical Centre, Ota, Nigeria

[4]Department of Regional Support Laboratory for Animal Influenza and other Transboundary Animal Diseases, National Veterinary Research Institute, Vom, Nigeria

[5]Department of Histopathology, Federal College of Veterinary and Medical Laboratory Technology, Vom, Nigeria

[6]Department of Medical Laboratory Science, Nnamdi Azikiwe University, Awka, Nigeria

[7]Department of Histopathology, University of Abuja Teaching Hospital, Gwagwalada, Nigeria

[8]Department of Pathology, Igbinedion University Teaching Hospital, Okada, Nigeria

ABSTRACT

Nigeria has continued to record a dramatic rise in the number of confirmed cases and fatalities of Coronavirus Diseas-2019 (COVID-19) since the first incidence was reported on the 27th of February, 2020. As of 25 August, 2020; Nigeria has the largest COVID-19 outbreak in the West-Africa sub-region (52,800), second to South-Africa (613,017) in the continent. In the absence of an effective and safe vaccine, accurate diagnosis in a timely fashion is very critical to the control of the pandemic. Diagnostic testing allows tracking of the virus, understanding its epidemiology, informing case management, interrupting and suppressing its transmission. Although several serologic methods are being developed and are currently being validated for the diagnosis of COVID-19, the only authorized testing platform for COVID-19 diagnosis in Nigeria as of now is nucleic acid detection in nasal swab, throat swab or other respiratory tract specimen using the Real time polymerase chain reaction (RT-PCR). Unfortunately, the country is not testing just enough due to its low molecular testing capacity. Lack of sufficient testing has been identified as the main reason we are not seeing the true picture of the outbreak in the country. In reality, the cumulative counts of COVID-19 cases may be ten times higher than reported, hence the need to scale-up the national molecular testing capacity amidst scarce resources. This review takes a look at the current practices, challenges and opportunities for molecular diagnosis of COVID-19 in Nigeria.

***Corresponding author**

Seyi Samson Enitan, Department of Medical Laboratory Science, Babcock University, Ilishan-Remo, Nigeria. E-mail Address: enitans@babcock.edu.ng; Phone no: +2348065483761.

Received: August 26 2020; **Accepted:** September 14, 2020; **Published:** September 27, 2020

Keywords: COVID-19, Molecular diagnosis, Challenges, Opportunities, Nigeria

Introduction

Cases of patients presenting with pneumonia of unknown origin was reported to the WHO in December, 2019 from the Wuhan City in the Republic of China [1-4]. Specimens were collected and laboratory test conducted. A novel coronavirus with very high propensity for spread was discovered. This virus was genetically related to the previously known and reported Severe Acute Respiratory Syndrome Coronavirus (SARS-COV) [5]. The international committee on the taxonomy of viruses named the novel coronavirus – Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), now known as the etiology of the Coronavirus Disease-19 (COVID-19) [6].Outbreaks of this novel coronavirus was subsequently reported in other countries with high human to human transmission occurring [7-8]. The impact of the novel virus globally has been enormous with close to 24 million people infected and 820,438 deaths recorded as of 25 August, 2020 and still counting in more than 216 countries and territories of the world [9]. In Africa, 1,014,834 cases have been reported with 20,787 fatalities. Nigeria has the largest COVID-19 outbreak in the West-Africa sub-region, second to South-Africa in the continent [10].

COVID-19 was first reported in Nigeria on the 27th of February, 2020. It was a case of 44 years old Italian who came into the country on February 24 and displayed symptoms of the disease, while visiting Lafarge Cement Company in Ewekoro, Ogun State. He has since been treated and discharged on 21 March,

2020 [11,12]. However, since the first incidence, the country has continued to record a dramatic rise in the number of confirmed cases. As of 12 midnight of 25 August, 2020, there were 52,800 confirmed COVID-19 cases with 1007 deaths recorded across all the 36 states, as well as the Federal Capital Territory (Abuja). 39,964 cases have been discharged, while 11,829 are currently undergoing treatment [13].

In an attempt to stall the rapid spread of the deadly virus, the World Health Organization (WHO) has declared diagnostic testing for COVID-19 as a critical intervention for tracking the virus, understanding its epidemiology, informing case management, interrupting and suppressing its transmission. The apex health organization strongly recommends testing of suspects for COVID-19 and the individuals who have been in contact with them in order to control the infection in a community [14]. Laboratory testing plays an important role in the identification of presumptive COVID-19 caused by the novel coronavirus [15]. Among the diagnostic testing options available are serological screening, virus isolation and cell culture and molecular technique, but not without one challenge or the other.Serological screening is bedeviled by some setbacks. Firstly, immunologic progression for COVID 19 is not yet fully established as of now. There is still a lot to learn about the disease in this regard. Secondly, the method is less sensitive. It is only appropriate for convalescent phase of the disease (not acute) and since antibodies do not peak before 9-11 days, it cannot be used to detect early infection. By the time antibody levels become detectable, the risk of transmitting the virus to others, with or without symptoms, is already high. This is the danger with using rapid diagnostic test (RDT) kits. People with negative results can still be positive and transmit this virus to others. Detecting COVID-19 after Day 14 of living with the virus does not help because the virus would have already spread to others by this time. Thirdly, the method is less specific due to the genetic relatedness between SARS-CoV-2 and other types of Coronaviruses. The possibility of cross-reactivity is responsible for the high reported cases of false positivity [16]. This has necessitated various calls for developing countries to stop the use of rapid diagnostic test kits for diagnosis of COVID-19 [17].

Virus isolation and cell culture on the other hand is not recommended as a routine diagnostic procedure. It requires the use of a biosafety level 3 and 4 facilities which is not readily available in most health institutions in Nigeria [18]. Also, the use of cell culture techniques using specialized vero cells takes between 3 to 5 days for cytopathic effects to occur; hence not practicable to establish the quick diagnosis of COVID-19 [4]. To this end, the nucleic acid amplification (NAAT) method, such as the real time reverse transcription polymerase chain reactions (RT-PCR) assay was adopted globally as the gold standard for the laboratory confirmation of COVID-19 because of its proven sensitivity and specificity. It has a major advantage of minimizing false-positive results associated with amplification product contamination as both amplification and analysis are done simultaneously in a closed system [19-22]. Other methods, however are currently being designed and are going through evaluation all over the world. These includes loop-mediated isothermal amplification, multiplex isothermal amplification coupled with microarray detection, and clustered regularly interspaced short palindromic repeats (CRISPR)-based assay [23].

In January 2020, the protocol for the real time RT-PCR for SARS-COV-2 was published [19]. This assay targeted the RNA-dependent-RNA polymerase (RdRp) gene, envelop (E) and the nucleocapsid (N) genes of the SARS-COV-2. The RdRp was highly sensitive with a yield of 3.8 RNA copies/reaction [20]. However, the probe used was a 'pan Sarbeco probe' which also detected other coronaviruses [16]. As at the time of development of this protocol, original SARS-COV-2 isolates were not available, hence synthetic nucleic acid technology was used to validate the assay. However, to improve the laboratory diagnosis of COVID-19, an improved real time reverse transcription PCR was developed and published in April, 2020 [20]. This improved assay targeted the RNA-dependent RNA polymerase (RdRp)/helicase (Hel), Spike (S), and Nucleocapsid (N) genes of the SARS-COV-2. Clinical specimen from laboratory confirmed COVID-19 patients were used for this assay. This RdRp/Hel assay was highly sensitive and specific for SARS-COV-2 and did not cross react with other human-pathogenic coronaviruses.

The quantitative real time reverse transcriptase polymerase chain reaction (RT-qPCR) is the gold standard for COVID-19 diagnosis and is currently being used in Nigeria as obtainable elsewhere. Serologic test could give supporting information but has not been recommended as a stand-alone test method for confirmation. The samples of choice are nasopharyngeal and oropharyngeal swab in ambulatory patients and sputum (if produced) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease. The lower respiratory materials have been reported to yield better results but for difficulty in sample collection. Sera samples are required for serological testing and seroprevalence studies [14-28].

The goal of COVID-19 testing is simply to detect infected individuals, so that they can be isolated and treated to prevent further spread. As such, the quality of the PCR results for COVID -19 is of utmost importance. This review takes a look at the current practices, challenges and opportunities in the molecular diagnosis of COVID-19 in Nigeria.

Current Practices

Proper collection of specimen at the right time from the right anatomic site in the pre-analytical stage is essential for a prompt and accurate molecular diagnosis of COVID-19. Appropriate measures are required to keep laboratory staff safe, while producing reliable test results in the analytic stage, using real-time reverse transcription-PCR (RT-PCR) assay. It is equally important that during the post-analytical stage, test results are carefully reported and interpreted. The rippling effect of a single undetected result as it affects isolation procedures is better imagined.

Pre-analytical Stage

The pre-analytical stage of COVID-19 testing consists of sample collection, transportation and storage.

Sample Collection

Respiratory samples with high amount of viral RNA yields are required for diagnosis of COVID-19. These include oropharyngeal, nasopharyngeal, sputum, saliva, and bronchial lavage fluid. The detection rate correlates with the amount of RNA in the sample [29]. Blood (serum/plasma) as well as autopsy/tissue samples can also be used [30]. The NCDC protocol for sample collection for the diagnosis of COVID-19 involves collection of one nasopharyngeal and one oropharyngeal swab as recommended for screening and early diagnosis [31]. Sputum samples in a sterile dry container with leak-proof screw cap can be collected from suspect cases presenting with cough. Bronchoalveolar lavage or tracheal aspirates are also used in rare cases [28]. However, nasopharyngeal swabs have become the preferred option as it is better tolerated by the patient, safer for the operator, and can reach

the correct site if performed accurately [22].

A recent study by Wang et al. shows that only 32% of oropharyngeal samples collected detected the SARS-CoV-2 RNA as opposed to 63% being detected by nasopharyngeal samples [16]. However, this seems to deal more with the frequency of isolation as it is apparent that both oropharyngeal and nasopharyngeal swabs were not collected from the same patients. Another factor limiting the collection of both oropharyngeal and nasopharyngeal swabs from the same patient, will be the difficulty and stress on the supply chain system at both national and international levels [22]. Sticking to the use of the nasopharyngeal swab alone (except where otherwise specified), will help prolong the use of available supplies. Proper collection of a nasopharyngeal swab specimen, is carried out by trained personnel, donning the appropriate personal protective equipment (PPE), and involves inserting the swab deeply into the nasal cavity, and kept in place for like 10 seconds while being twirled 3 times before retrieving it [28].

Sample Transportation and Storage

The swabs are placed in a tube containing 2-3mls of virus preservative solution or tissue culture solution or isotonic saline solution including phosphate buffer saline (PBS), Hanks, viral transport media (VTM) and viral RNA isolation buffer, sealed with para film and inserted into a Ziploc bag [28] for rapid transportation to the clinical molecular laboratory, in this case, one of the NCDC laboratory network, ideally under refrigerated conditions at 4°C temperature or on dry ice [32]. Specimen can be stored at 4°C for 24 hours and -70°C or below (or temporarily at -20°C where this is not available). Repeated freezing and thawing should be avoided. Because of the very high transmissibility of the virus from human to human, the timing of testing for COVID-19 suspected case is very important. Early detection of the disease can facilitate the quick conduct of contact tracing, isolation and treatment. Hence, there should be no delay in sample processing.

Analytical Stage

Current knowledge reveals that SARS-CoV-2 is a Biosafety Level (BSL)-4 pathogen because of its high transmissibility and infectivity; hence appropriate containment is needed during RNA extraction at the analytical stage to keep healthcare workers safe [18]. However, in resource limited countries, where the Maximum Containment laboratory, Level 4 is lacking, it is required that detection of SARS-CoV-2 in suspected clinical specimen is performed in Biosafety Level 3 (BSL-3) facility or at least a BSL-2 as required by International Standard Organization under appropriate conditions and by trained laboratory personnel [33]
.

Nucleic acid extraction

There are several methods available to isolate viral RNA for the extraction of the viral genomic material from the sample [34]. Since detection rate correlates with the amount of RNA in the sample, it is important to choose samples that are likely to yield very high RNA virus shedding. High yields of viral shedding are found in respiratory samples. Considerations must also be given to the method/ technique of inactivation whether heat or chemical inactivation process because this have an effect in the integrity of the RNA product [35].

Conventional Reverse transcriptase PCR

This laboratory technique combines reverse transcription of RNA into DNA and amplification of these specific DNA using polymerase chain reaction [36]. In most living organisms, it is DNA that is transcribed to produce RNA, so when an RNA virus transcribes RNA to DNA, the process is called reverse transcription. The complementary DNA (cDNA) produced in this way can be amplified by the process of polymerase chain reaction to increase the amount of cDNA, thereby making it easier to measure. The genetic material (RNA) of the virus is first reverse transcribed to yield a complementary DNA (cDNA) transcript from the targeted probe and thereafter a thermostable DNA polymerase is used to amplify the specific gene fragment by means of the polymerase chain reaction (PCR). The targeted genes for the SARS-COV-2 are the E, N and RdRp gene fragments as earlier mentioned. During the PCR reaction, the DNA polymerase cleaves the fluorescent probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes the target DNA. This produce a signaling intensity which increases with each cycle of RT-PCR. This fluorescence intensity provides information about the quantity of viral RNA present in the sample. The PCR cycle at which an increase in the fluorescence signal is detected initially (ct) is proportional to the amount of the specific PCR product.

Real Time Reverse transcriptase PCR

The fluorescent signal generated by the cleaved reporter dye can be monitored real time by an improved PCR system. Monitoring the fluorescence intensities in real time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification. This technique allows scientists to see the results almost immediately, while the process is still ongoing, unlike the conventional RT-PCR which provides results at the end of the process. Hence the test is said to be done in 'real-time'. The real time PCR optical unit measures the emitted fluorescence. The detection of the amplified fragment is performed in the fluorimeter channels – FAM, HEX/VIC/JOE and the Cal Red 610/ROX/TEXAS RED with fluorescent quencher [35-37].There are also emerging techniques like the Loop-mediated Isothermal Amplification (LAMP) technique, which is carried out at a constant temperature without requiring a thermocycler. However, this requires some form of validation. Further characterization of the virus can be achieved through sequencing of the viral genome using the conventional or the next generation sequencing methods, although this requires higher technical skills [38].

Interpretation of Test Result

In the interpretation of COVID-19 test result, the controls (i.e., the positive, negative and internal controls) must be valid. Viral RNA in sample is measured by the cycle threshold (Ct) which is the number of replication cycles required to produce a fluorescent signal, with lower Ct values representing higher viral RNA loads. A Ct value less than 40 is clinically reported as PCR positive. This positivity starts to drop by week 3 and subsequently becomes undetectable. In most individuals with symptomatic COVID-19 infection, viral RNA in the nasopharyngeal swab is detectable as early as day 1 of symptoms and peaks within the first week of symptom onset. The Ct values obtained in severely ill hospitalized patients are lower than the Ct values of mild cases, and PCR positivity may persist beyond 3 weeks after illness onset when most mild cases will yield a negative result [39].

Post-Analytical Stage

The post-analytical stage is equally as important as pre-analytical and analytical stages described above. It consists of the reporting, checking (verifying), timeliness, and interpretation of COVID-19 test results. It is very vital for COVID-19 test results to be properly reported, documented, and verified before being issued or dispatched. Test results must be clearly and informatively presented using the approved guidelines for reporting molecular test for COVID-19. It is very important that test results are reported correctly since positive results have serious public health

implications. In addition, reporting of COVID-19 test results must be standardized to allow uniformity across the laboratory network in the country. The method of reporting test results must be clearly written in the Standard Operating Procedure (SOP). The specimen examined must be stated and reports must be written (typed) clearly and neatly using standardized forms. All test results must be double-checked before they are issued or dispatched. Clerical error must be avoided by all means. There must be no disparity in the patient's name and laboratory identification number of test sample(s). Misidentifying a patient can be avoided by compared information on the request form with the test result to be issued.

Furthermore, the NCDC laboratory network must put in place a system (if not already available) for monitoring whether test results are issued/dispatched early enough to influence both clinical and public health decision making. Testing sites are expected to electronically transmit data daily, within 24 hours of test completion, to the NCDC database. Data to be transmitted should include: name of testing laboratory, specimen ID, Epid Number, Name of patient, gender, age, specimen source, date test was ordered, date specimen was collected, date specimen was received at the laboratory, date specimen was tested, nature of test (initial, repeat or follow-up) and the test result proper: CT Values for qRT-PCR Target (E-GENE), EAV(IC), RDRP-GENE, N-GENE, ORF 1-GENE and the final result interpretation (Positive, Negative of discarded). This calls for proper collection and documentation of patients' demographic information by the testing laboratories [40- 41].

Challenges of COVID-19 Testing in Nigeria

Diagnostic testing for COVID-19 has been very valuable in the identification and confirmation of suspected cases in Nigeria, but has also met with several challenges. These include:

Low Testing Capacity

According to the Director-General of WHO, testing remain one of the key measures to curb the spread of the deadly virus. Unfortunately, many resource limited countries, Nigeria inclusive, have low testing capacity (facilities, equipment and human resources). The number of cases reported so far by any country is a reflection of the testing capacity of such country, and not the actual current burden of the infection [42]. In fact, maps that do not show any cases of COVID-19, are an indication of lack of testing, rather than absence of the virus in such community. According to WHO interim guidance, not having laboratory-confirmed cases does not suggest that a country is free from COVID-19 and can be a sign of insufficient testing and surveillance [43]. Generally, the number of tests performed is different from the number of individuals tested. The reason for this is that the same person is testedmore than once. Some countries report only tests performed, others report the number of individuals tested, while some report both [44].

As earlier mentioned, the real time RT-PCR is the WHO recommended method of analysis [23, 45] which can be supplemented by serology (yet to be adopted in Nigeria). Initial diagnosis in a country with no recorded case requires random deep-sequencing methods such as next-generation sequencing or metagenomics next-generation sequencing. This will further be used in monitoring mutations in the field strain. Shortly before the virus was detected in Nigeria, the Nigeria center for Disease control (NCDC) had activated four testing laboratories in her molecular laboratory network. Five months down the line, 61 molecular laboratories have been activated across 31 states of the federation, leveraging on the already existing Gene-Xpert capacity for HIV and Tuberculosis diagnosis (Figure 1). A few states are yet to have any of these testing laboratories, while some states are yet to have their laboratories accredited, and a few of them have more than one accredited testing centers. The country is currently carrying out between 3,000-6,000 COVID-19 tests daily, even though the available testing infrastructure can conveniently carry out about 15,000 tests per day [46].

The truth is that in Nigeria, we are not testing anywhere near enough. With a population of about 200 million, less than 400,000 (388,346) samples have so far been tested, while South Africa with a population of 59 million has tested over 3.5 million (3,578,836) samples as of 25 August, 2020 [13, 47]. Our low testing capacity is largely responsible for the under-reporting of COVID-19 in the country (52,800 confirmed cases) (Figure 2), compared to the 613,017 reported in South-Africa. Even with the robust testing capacity of the United States of America (about 74 million tests so far with a population of 330 million), a recent study shows that COVID-19 cases may be 10 times higher than reported in the US [48], as only severe cases and priority groups are getting tested. In Nigeria, like elsewhere, the early-stage asymptomatic characteristic of the virus has permitted silent transmission which is a further concern to identify and test those infected [49].

Low testing capacity has continued to undermine the fight against the virus in Nigeria. It specifically hampers and delays the ease with which samples are collected, sent and results generated for effective treatment. While the use of GeneXpert has been activated, we will like to mention here that not all GeneXpert machines are suitable for COVID-19 testing.Going further, shortfalls in manpower with the required expertise is central to the challenge of low testing capacity. Molecular diagnostic laboratories cannot be operated without the services of highly skilled, trained and licensed medical laboratory professionals [43]. In Nigeria, there are many trained personnel who have failed to perfect their hands on skills requires for molecular testing because of the non-availability of machines and reagents to work with. It becomes very challenging to begin to train and perfect skills in the face of a pandemic. This diminishes output at the start only to pick up later. This may however reduce the life span of machines as there may be little errors committed in the course of operation that may be deleterious to the machines.

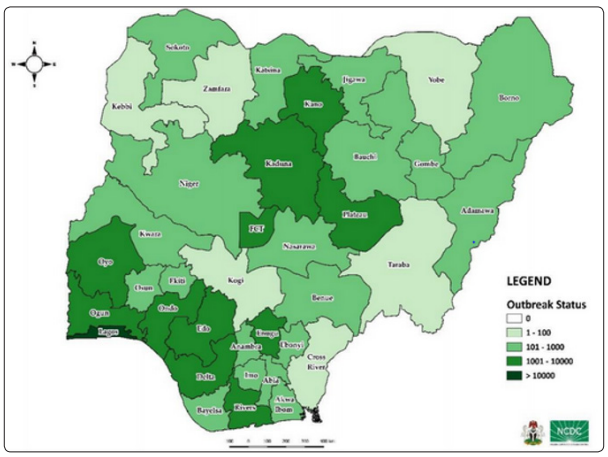

Figure 1: Map of Nigeria showing 36 states and FCT affected by COVID-19 as of August 25, 2020.

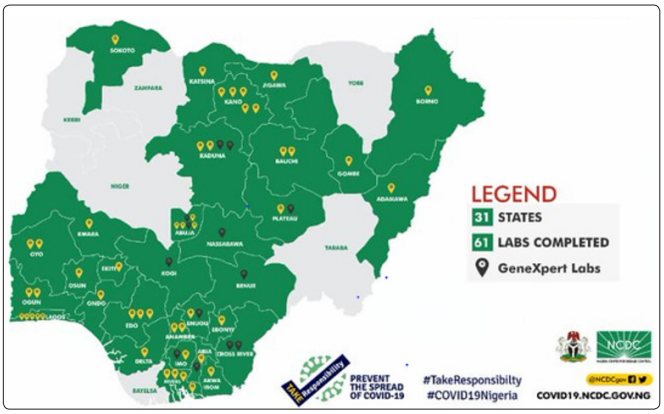

Figure 2: NCDC Molecular Laboratory Network as of 31 July, 2020

Inadequate Containment Facility

The Coronavirus (SARS-CoV-2) falls into the Risk Group 4 category, just like it counterparts: SARS-CoV-1 and MERS. The pathogens in this group are deadly and offer a high risk to the laboratory worker and to the community. They can cause serious disease and are readily transmitted from one individual to another. The Maximum Containment laboratory, Level 4 is intended for work with viruses in Risk Group 4, for which the most-strict safety precautions are necessary. However, in resource limited countries, where the Maximum Containment laboratory, Level 4 is lacking, it is required that laboratories involved in COVID-19 non-propagative diagnosis operate a biosafety level 2 (BSL-2) and level 3 (BSL-3) for those involved in cultures. This also poses a great challenge to COVID-19 diagnosis in Nigeria as very few laboratories operate certified BSL-2. This goes to say many personnel have been working under very risky environment even before the advent of COVID-19 pandemic as there are other routine diagnoses done in-country which also requires BSL-2 [18].

High Cost of testing

High cost of testing is synonymous with low testing capacity in resource limited countries. This is so because molecular testing of COVID-19 requires high profile and very expensive test kits and equipment such as the PCR machine, as well as adequately equipped Biosafety Laboratories (at least BSL-2 labs), making the cost of large-scale testing less affordable by low and middle income countries (LMICs) including Nigeria. Worst still, the test kits and equipment are in gross short supply within the country. The situation is further confounded by the fact that the equipment are in high demand all over the world. Reagents and consumables needed to run these machines suffer the same fate of short supplies. The total or partial lockdown of producing countries has continued to affect the importation of these equipment and consumables gents into the country. Unfortunately, none of these machines or consumables are produced in Nigeria, a situation that further increases the cost of testing. An example of critical reagents are the extraction kits. While these challenges are global, countries with a robust research capacities have significantly higher test rates as private and academic research institutions in such countries also assist in testing [50].

Although the Federal Government of Nigeria makes molecular testing for COVID-19 free, majority of the populace are unable to access it due to low testing capacity relative to the population size; hence, the permission granted to some accredited private laboratories to test for COVID-19. Price range for COVID-19 testing by accredited private laboratories in Nigeria is currently between ₦25,000 – ₦75,000 ($50 - $150) per test. The concern here is this, how many Nigerians can afford this? A report by the World Poverty Clock, shows that Nigeria has the largest extreme poverty population with 86.9 million (50%) living below the poverty line (i.e, less than US$1.90 per day). Currently, the data stay at 48% with many unable to go for routine medical check-up and laboratory testing due to cost [51-52].Like in other LMICs, many Nigerians do not even have a health insurance. The fear of the outrageous cost of testing for COVID-19 and underlying conditions, together with the cost of isolation amidst the current economic crisis induced by the pandemic is keeping many people away from getting tested. To make the matter worse, unaccredited private laboratories without the necessary requirements for COVID-19 testing are busy taking advantage of the current situation, exploiting the masses, all for financial gain. The reality on ground is that not everyone is getting tested (only a selected few are getting tested) or testing is done too late. This seriously undermine the fight against the pandemic. Under-testing will no doubt, obstructs the proper understanding of epidemiology of COVID-19, thereby increasing the risk of undetected community spread [53]. This approach has also been blamed for contributing to the continued spread of COVID-19 as undiagnosed cases drive persistent community transmission [54]. Furthermore, a blinded view of COVID-19 means that government responses may not be commensurate with the actual situation [53].

Difficulty in Sample Collection and Transportation

Many suspects of COVID-19 have been reported to be unwilling to make sample available for testing due to the fear of been stigmatized. COVID-19 has been associated with stigmatization and derogatory labels in Nigeria like elsewhere around the globe [55, 56]. And as a result, many potentially exposed persons are afraid to come out to get tested and receive treatment if found positive. They would rather die in silence, while they constitute a silent, but potent reservoir maintaining the human-to-human transmission of the virus within the society. To make matter worse, when COVID-19 associated signs and symptoms begin to appear, they instead engage in self-medication including the use of unproven herbal remedy. As a matter of concern, some exposed individuals have refused to quarantine, while some confirmed cases have been reported to flee isolation centres in different parts of the country including Oyo, Kano, Bauchi, Taraba, Gombe and Delta states amongst others [57]. This development has further impacted negatively on the fight against the pandemic. Currently, as of 21 August, 2020, a total of 11,585 persons are of interest to the NCDC for the purpose of COVID-19 testing. More is needed to be done with regard to contact tracing as majority of those with travel history to epicenter (National and International) of the virus either provided incomplete or false contact information.

Furthermore; the decision on what sample to collect, when to collect (right timing), and transport samples promptly and under the right condition pose serious challenge. It has been documented that a single negative test result especially from the upper respiratory material does not exclude an individual from possibly being infected with the virus. Viral load is usually at its highest within 5-6 days [31-60]. Moving to the left or the right reduces the tendency of detecting the virus. This implies that there is a tendency of not detecting the virus in an infected person when samples are taken too early or too late. The lower respiratory materials are suggestively preferred as the disease advances. Despite better results obtained from lower respiratory materials, the nasopharyngeal and oropharyngeal swabs are the most often used because of the convenience and inherent control in collection of nasopharyngeal swab. Based on recommendations

the oropharyngeal and nasopharyngeal swab are used together in the same viral transport medium at the same time in Nigeria to avoid missing any case [31,45,61]. Though nasopharyngeal and oropharyngeal swabs may be sufficient for diagnosis at periods when the viral load is at its peak, late detection and monitoring of individuals with severe COVID-19 pneumonia requires the use of lower respiratory materials such as sputum or a bronchooalveolar lavage, which may be difficult to collect in some patients [57, 62, 63]. Also, time is a crucial factor affecting COVID-19 molecular testing when lives can be saved and deaths averted. Samples need to be transported to the laboratory, and the test itself takes 1 to 3 hours after they must have undergone RNA extraction before the test can be run, adding another 1 to 2 hours. Hundreds of samples can be processed at once depending on the laboratory capacity. RT-PCR is labour intensive, severely constraining the capacity for quick turnaround times from sample collection to results transmission which might take days in getting results [64]. As a result, laboratory testing of suspected cases is characterized by long wait periods and an exponential increase in demand for tests [65]. These samples are carefully packaged and transported to the distant testing sites under conditions that protect sample integrity. This multi-step, intensive process results in huge backlogs, severely slowing suspect case testing and limiting capacity for mass testing.

Worst still, most of the Nigerian hinterland and rural areas are bedeviled with poor road network, hence non accessible. To this end, sample collection and transportation becomes very difficult. Consequently, many Nigerians do not get their samples collected for testing. And where samples were successfully collected, delay in sample transportation, processing and release of test results have been reported due to poor road network.

Poor Power supply for Sample Storage and Analysis

The need to prioritize power supply to health care facilities amidst the COVID-19 pandemic is another major challenge. For most of the high technology equipment use for COVID-19 molecular testing to function optimally, it is critical to have an optimal environment including a stable and efficient power supply. Unfortunately, the epileptic nature of power supply in Nigeria places an enormous burden on molecular diagnosis because to set up any molecular laboratory, huge investment on power supply would need to be made if the laboratory must be functional. Although the Federal government through the electricity distribution companies (DisCos) promised to supply electricity to Nigerians free of charge for two months. This never materialize, rather, cases of total power outage amidst the pandemic have been reported here and there with consumers paying for power they never enjoy [66- 67].

Lack of motivation and protection for the healthcare workers

Poor remunerations and poor working conditions compounded with excess workload is another clog in the wheels of the efforts towards achieving a robust and resilient diagnosis of COVID-19 in Nigeria [68]. Compared with their counterparts elsewhere around the globe, Nigerian health professionals appears to be lest paid in terms of hazard allowance and life insurance. Lack of Personal Protection Equipment (PPE), as well as lack of training on infection prevention and control, particularly for rural healthcare workers are primordial issues killing the morale of the frontline armies in the fight against the deadly virus in Nigeria. There has been global shortage of materials needed to run the end-to-end testing process at full capacity, particularly the reagents that help to ensure high levels of sensitivity and specificity for these tests. More worrisome is the shortage in Personal Protection Equipment (such as the N95 respirator, face masks, shields and isolation gowns among others) necessary to ensure the safety of healthcare workers, owing to the global demand for these items. Lack of these supplies have hinder testing and escalate the spread of the virus. Around 6% of the COVID-19 cases in the country composed of healthcare workers, with some of them working in private clinics without the necessary training and necessary precautions. They have not only infected themselves, but have also become a source of infection to their families. Many exposed health workers (Medical Laboratory Scientists inclusive) are currently either in quarantine or isolation; while others have lost their lives since the fight against COVID-19 started. Consequent upon these, workflow is being disrupted with possible discontinuity of healthcare services in some cases [18- 69].

Industrial disharmony in the health sector

The unending professional rivalry and struggle for dominance, autonomy and recognition among the healthcare workers in Nigeria is further undermining the fight against the pandemic. Doctors and nurses are so visible at the front lines and are being applauded for the gallant role they are playing in the recovery of hundreds of thousands of COVID-19 patients, but not so with the laboratory personnel despite the critical role play in the fight against the pandemic. An effective and timely diagnostics approach is central and necessary for the successful containment of any outbreak and the Medical Laboratory Scientists (MLS) are at the fore-front. They are the ones testing clinical specimens from infected and clinically recovered patients. As disease detectives, their role in the fight against the COVID-19 pandemic include, but not limited to: diagnosis, monitoring, confirmation of recovery, discovery and development of vaccines, safety and efficacy testing of broad-spectrum antiviral agents, validation of testing protocols and testing kits, offering of advisories to guide government policy on containment at all levels amongst others [15]. Unfortunately, the laboratory personnel appear not to be recognized by the public since the nature of their works keeps them in perpetual obscurity. This raises great agitations that disrupt dedication and collaborative efforts to the fight against the pandemic. There have been instances of verbal and non-verbal confrontation between the Pathologists and the MLS regarding the headship of the laboratory. The Association of Medical Laboratory Scientists of Nigeria (AMLSN) has once expressed deep concerns over the alleged exclusion of some of their members with expertise in molecular diagnosis in the ongoing COVID-19 testing across the country. Such professional rivalry is unhealthy for the Nigerian fragile health system amidst the pandemic.

The need to optimizing test procedure

With a novel pathogen like SARS-CoV-2, just having the viral nucleic acids available to test if the assay is working has been the first challenge which emanates from the nature of RT-PCR tests. RT-PCR can be performed in either a one-step or a two-step assay. In a one-step assay, reverse transcription and PCR amplification are combined into one reaction. This assay setup can provide rapid and reproducible results for high-throughput analysis. The challenge is the difficulty in optimizing the reverse transcription and amplification steps as they occur at the same time, which leads to lower target amplicon generation. In the two-step assay, the reaction is done sequentially in separate tubes. This assay format is more sensitive than the one-step assay, but it is more time-consuming and requires optimizing additional parameters such as specialized reagents called primers and probes are necessary for the test to be run for detecting COVID-19 cases when testing upper respiratory tract, saliva, and plasma specimens from patients [27,70,71] .

Limitations to interpretation of test

RT-PCR is prone to false negative and false positive results. False negatives could be due to insufficient samples, error during sampling, timing of sampling and testing (viral RNA levels may differ during clinical course) and technical/human errors during the PCR test. The different kits from different manufacturers needs to be validated in each laboratory before being used for diagnosis. False positives on the other hand can arise due to certain factors including: surge in the outbreak, resulting in increase in sample volume to the laboratory for testing; human errors, variability in test methods and personnel technical skills and contamination.

RT-PCR uses Primers

A variation in the viral RNA sequence can result in false negative results because mutations in the primer, probe target regions in the SARS-CoV-2 genome (though rare as conserved regions are choice targets) may cause a mismatch of primer and probe target region resulting in false negative result. An attempt to overcome this challenge is the use of multiple target gene amplification approach thus avoiding invalid results. Some cases of genetic diversity and rapid evolution of the novel SARS-CoV-2 have been reported [72-74].

Sensitivity of RT-PCR is not 100%

Its accuracy is dependent on Laboratory Practice Standards and personnel skill in the relevant technical and safety procedure.

1. Timing and anatomical site of sample collection.
   Optimum sample timing is yet to be fully determined. Some research has recorded a peek viral content at day 5-6 post onset of symptoms in both lower respiratory tract and upper respiratory tract. In this case low viral content was already detected as early as day 2 -3 and viral detection at low level continued until 13-14days. While in another case where viral presence was not detected until day 14 and became undetectable by day 18-20, but resurfaced on day 25. The initial low viral detection was lower than the first case [72]. As for anatomical sampling site, a study suggest the best sample is sputum and next is nasopharyngeal swab in ambulating patients [75]. The authors discouraged the use of throat swab for diagnosis of COVID-19. Bronchoalveolar lavage fluid is recommended for diagnosis of severe cases. This is limited by the fact that it requires suction tools, expert operator and invasive procedure [26-28]. Sampling must be by dacron or Polyester flocked swab
2. Presence of amplification inhibitor in sample or inadequate viral content due to wrong sampling, transportation and handling.
3. False positive results are usually indicative of sample contamination. This calls for regular inclusion of internal controls which could help identify specimens containing substances that could interfere with nucleic acid extraction and amplification.

Interpretation of results

At present, interpretations are made based the following conditions If a case test positive both nucleocapsid proteins N1 and N2 (Targets in the CDC assay), it is considered laboratory confirmed positive case. The cycle threshold (CT) valve <40 is the indicator of positivity while CT valves ≥40 is indicative of a negative result.If CT valves for one of the two nucleocapsid proteins (N1 or N2) is ≥40, it is considered as indeterminant and requires a confirmation a rerun of the test [76].China has however adopted the use of three targets where if any two are positive or more, it is considered confirmed positive [77]. A study carried out in 2004 on SARS patients indicated that though viral RNA was present in some patients for 30 days or more after the onset of symptoms, none of these individuals had a positive viral culture from 22 days after the onset of the illness. Though not specific to the corona virus that causes COVID -19, this could be an indication that though viral RNA may be detected using the RT-PCR method, this virus may not be alive and transmissible [78]. The implications of this can be an area of further studies, as follow up on therapeutic response using RT-PCR may give a wrong interpretation [59-77]. The issue of false negatives may be responsible for the many situations where initial test results have come out negative and upon a retest have been diagnosed as positive, as with the case of a state governor recently. It is possible that those who tested negative initially, may have had a higher cycle threshold, with mild or no symptoms, thus the early detection of production of antibodies may be useful in validating RT-PCR results [79], as antibodies take even longer to develop in asymptomatic people. Another study by Li et al. showed less than 40% with positive RT-PCR test among 610 patients presenting with a clinical diagnosis and a typical CT scan appearance of COVID-19 pneumonia [62] . Such 'negative' cases with resolutions of COVID-19 pneumonia may still be shedding viable corona virus and are likely to infect others [80].

Despite the foregoing, tests for COVID-19 are only being restricted to symptomatic cases because of difficulties with access to testing facilities, logistics and lack of testing materials, and patients presenting with respiratory symptoms do not get the proper treatment, until COVID-19 diagnosis is ruled out, a situation that can be fatal for the patient. Prompt identification of COVID-19, alongside other concurrent bacterial and viral diseases will greatly improve management and treatment outcomes. In as much as testing is needed to identify active cases, adequate testing is equally needed on COVID-19 recovered cases to show viral absence before their plasma can be collected by donor center for further studies. Bottlenecks in the supply chain may equally be a contributing factor to having patients discharged after one negative test as opposed to two negative results of 14 days' interval that was obtainable in the past. There have been reports, though unconfirmed, of patients who hitherto have recovered from COVID-19, being positive for RT-PCR throat swab after 28days of being symptomatic. Therefore, monitoring the safety of blood and plasma donation is very important to be sure that the virus detected is not infectious [78].

The application of results in the monitoring of patient's response to treatment and determination of infectivity as a guide to knowing when patients are qualified for release from isolation is also critical. The practice has been to wait until at least two consecutive laboratory tests result are negative. This approach has been reagent exerting as each isolated patient may have to get several turns of test thereby using up some of the reagents that should have been used to test others. Recently, based on the findings from Singapore, which indicated that despite a positive RNA test by the 13 day, patients are no longer infective, the new NCDC guideline provides for a discharge for any patient 10day post-onset of symptoms that has elicited at least 2 days of no symptoms irrespective of a positive RNA test [81].

Opportunities and Recommendations

Beyond ensuring that everyone who meets the case definition gets tested, there is need for targeted large-scale testing and this can only be achieved through a more rapid, accurate and a□ordable diagnostic testing approach and scaling up laboratory testing capacity.

As more funds become available, there is need to expand the existing NCDC laboratory network for molecular testing of COVID-19. The ongoing leverage capacity within high-throughput HIV molecular testing laboratories and utilization of point of care tuberculosis testing geneXpert machines for COVID-19 testing is well recommended. We also need to establish more static and mobile diagnostic laboratories for rapid response.

Since testing is a critical component of the pandemic response, we need to increase the combined national testing capacity from the current 3,000-6,000 tests per day to a minimum of 20,000 tests per day across the country if substantial progress must be made in combating the pandemic. Hence, the country must strive to utilize its existing testing capacity to the fullest, as only 40 percent of it is being deployed at the moment. Despite the current turn-around time of 3-6 hours while using real time RT PCR, it is quite evident that with an increase in the rate of confirmed cases, there is a need for more a shorter turn-around time. To this end, private partnership with already existing molecular laboratories having biosafety level 2 or 3 should be continuously encouraged, as exemplified in the partnership of 54 gene laboratory with FCMB and Ogun State government in the provision of mobile molecular laboratories.

As global shortages of diagnostic kits and laboratory consumables increasingly impacts the optimal functionality of the laboratory system in Nigeria and across the world, an adaptive testing strategy is required to ensure that the most vulnerable persons (e.g. children, pregnant women, elderly etc.), those at elevated risk (e.g. patients with liver and kidney disorders), and those with super spreading potential (e.g. commercial sex workers, commercial motorcycle operators etc.) have access to testing. Hence the need for enhanced technical input and logistic support, home and abroad . More human and materials resources should be committed into mass testing to survey the population, to learn more about the virus and contribute to research and development.We need to develop a resilient testing algorithm with emphasis on accuracy, speed and availability. To do this, we may need to consider pool testing to both reduce cost and reduce the duration from sample collection to result generation. Pooling samples is scientific and has been reported to boost the COVID-19 testing in many countries [82]. In the case of Ghana for example, each pool has 10 samples and 100 pools are tested at a time. Instead of testing one person at a time, samples from multiple individuals are put together and tested as one pool. If the pooled test comes back negative, everyone in the pool is declared negative. But if it is positive, each member of the pool is then retested individually for the infected person to be identified. Pooled sampling does not only scale up PCR testing capacity rapidly, it also utilizes less testing reagents and shortened the results waiting time from two (2) to around six (6) days, helping to shift the backlog of samples for testing that have built up in the laboratories, and relieving overcrowded isolation centres. To this end, Ghana has conducted over 370 000 tests between March and mid-July, 2020 making it one of the countries in the WHO Africa Region with the most tests per 100,000 populations [83]. This testing strategies is very cost effective as 370,000 pool sampled tests would cost about USD 7.4 million ($20 per test), instead of USD 74 million if samples were to be tested individually. Unfortunately, Nigeria has been unable to achieve its target of 2 million tests in 3 months. To catch up with her African counterparts (South Africa and Ghana), Nigeria needs to adopts pooled samples testing strategies.Extensive training of the teaming medical laboratory workforce is paramount to the execution of this project. Actively including personnel in both public and private sectors will make it possible to deploy more man-power to the newly established molecular diagnostic laboratories. Procurement and distribution of supplies to laboratories should remain a priority focus in the testing expansion strategy as part of the overall infection control measures.The supply of certified kits cannot be over-emphasized. With a looming shortage in the supply of these molecular testing kits, our indigenous pharmaceutical, scientific and manufacturing companies should be greatly encouraged to pursue international best practices in the rapid development of integrated, random-access, point-of-care molecular devices for the accurate diagnosis of SARS-CoV-2 infections through friendly policies and investment. Care should however be taken to avoid potential cross-reaction with other endemic coronaviruses as well as potential genetic drift of SARS-CoV-2. The shorter turnaround-time associated with these point of care molecular tests will be very important for real-time patient management and infection control decisions. These assays are safe, simple, and fast and can be used to test for as many cases as possible.

Furthermore, the use of syndromic testing as a tool to complement COVID-19 diagnosis will be a welcome development. This will aid the rapid diagnosis of other bacterial and viral pathogens presenting with symptoms similar to COVID-19, thus reducing the burden on the isolation centers and initiate prompt monitoring, intervention and treatment.Bottle necks associated with collection and transportation of samples over long distances can be surmounted with the advent and deployment of RDT kits. Though there is none certified at present, this is an area to be critically looked into. This will also foster prompt diagnosis which is pivotal to the effective management of any disease. The rapidity and ease of diagnosis has in the past very useful in the fight against epidemics and pandemics like HIV and malaria. The discovery and use of high efficacy rapid diagnostic test (RDT) kits could help fight the current pandemic as it will enhance a quick, convenient and wider coverage of testing. To this end, we need to explore the role of antigen and antibody tests in the future to enable us understand the rate of infection, and how the virus is spreading across the country (sero-prevalence). This will enable an assessment of the impact of measures taken so far to contain the virus, to inform current and future actions.

Finally, the safety of health personnel is to be taken very seriously. Many health personnel in the frontline of the fight against COVID-19 have been reportedly infected and some have lost their lives due to lack and inadequate personal protective equipment (PPE), poor hazard allowances and life insurances [1, 83].

Conclusion

No doubt, the novel coronavirus has come to stay with us until a safe and effective vaccine is made available, accessible and affordable. In the absence of an established cure or preventive vaccine, diagnostic testing remains an essential response strategy to interrupt the transmission of the deadly virus. Lacking of sufficient testing is the main reason we are not seeing the true picture of the disease in the country. All infected individuals within the population must be properly identified using the approved diagnostic method. Although molecular (PCR) assay may be prone to false negativity and false positivity, it still provides the most reliable results (in terms of specificity & sensitivity), to detect active infection to inform public health response activities currently in Nigeria. To this end, we need to increase our national testing capacity by continuously expand the existing NCDC molecular laboratory network, without failing to ensure the safety, training and re-training of our healthcare workers on infection prevention and control to reduce their risk of exposure. It is also important that the educational curricular for courses on infectious

diseases be revised with the intention of meeting the paradigm shift in disease diagnosis, prevention and control in the country. Accurate, real-time actionable testing data is crucial in the fight against this virus. This will help policymakers in making timely decisions necessary for containment, hence the need for us to optimize the national COVID-19 electronic data management system for better performance. Accurate and reliable data on COVID-19 will enable us to understand how the pandemic is progressing and how to respond appropriately to it.

Competing Interests

Authors have declared that no competing interests exist.

Funding

This paper was not funded.

References

1. Enitan SS, Ibeh IN, Oluremi AS, Olayanju AO, Itodo GE (2020) The 2019 Novel Coronavirus Outbreak: Current Crises, Controversies and Global Strategies to Prevent a Pandemic. International Journal of Pathogen Research 4: 1-16.
2. Lu H, Stratton CW, Tang YW (2020) Outbreak of pneumonia of unknown etiology in Wuhan China: the mystery and the miracle. J Med Virol 92: 401-402.
3. Ralph R, Lew J, Zeng T, Francis M, Xue B, et al. (2020) CoV (Wuhan virus), a novel Coronavirus: human-to-human transmission, travel-related cases, and vaccine readiness. J Infect Dev Ctries 14: 3-17.
4. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, et al. (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579: 270-273.
5. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF (2020) The proximal origin of SARS-CoV-2. Nat Med 26: 450-452.
6. Gorbalenya AE, Baker SC, Baric RS, De Groot RJ, Drosten C, et al. (2020) The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 5: 536-544.
7. Sifuentes-Rodríguez E, Palacios-Reyes D (2020) COVID-19: The outbreak caused by a new coronavirus. COVID-19: la epidemia causada por un nuevo coronavirus. Bol Med Hosp Infant Mex 77: 47-53.
8. World Health Organization (WHO) Coronavirus disease 2019 (COVID-19) situation report–23. Geneva, Switzerland:https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200212-sitrep-20200223-ncov.pdf?sfvrsn=20200241e20200219fb20200278 20200212 [16 June, 2020].
9. JHU-CSSE (2020) Coronavirus: COVID-19 Global Cases by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University. https://gisanddata.maps.arcgis.com/app s/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6.
10. World Health Organization (WHO) COVID-19 in the WHO African Region. ttps://who.maps.arcgis.com/apps/opsdashboard/index.html#/0c9b3a8b68d0437a8cf28581e9c063a9 (Accessed on August 26, 2020).
11. Nigeria Centre for Disease Control (NCDC). "First case of Corona virus disease confirmed in Nigeria. Official Website of the Nigeria Centre for Disease Control." Available: https://ncdc.gov.ng/news/227/first-case-ofcorona-virus-disease-confirmed-in-nigeria.
12. Ibeh IN, Enitan SS, Akele RY, Isitua CC, Omorodion F (2020) Global Impacts and Nigeria Responsiveness to the COVID-19 Pandemic. International Journal of Healthcare and Medical Sciences 6: 27-45.
13. Nigeria Centre for Disease Control (NCDC) "Covid-19 situation report situation report 179 August 25 2020_170420_16. Official Website of the Nigeria Centre for Disease Control." Available: https://ncdc.gov.ng/diseases/sitreps/?cat=14&name=An%20update%20of%20COVID-19%20outbreak%20in%20Nigeria.
14. World Health Organization (WHO) Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans. March 22, 2020. Retrieved from https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance.
15. Ibeh IN, Enitan SS, Akele RY, Isitua CC (2020) A Review of the COVID-19 Pandemic and the Role of Medical Laboratory Scientists in containment. J Med Lab Sci 30: 68-89.
16. Wang C, Liu Z, Chen Z, Huang X, Xu M, et al. (2020) The establishment of reference sequence for SARS-COV-2 and variation analysis. J. med. Virol 92: 667-674.
17. Umaru F A (2020|) Scaling up testing for COVID-19 in Africa: Responding to the pandemic in ways that strengthen health systems. Afr J Lab Med 9: 1244.
18. Itodo GE, Enitan SS, Oyekale AO, Agunsoye CJ, Asukwo UF, et al. (2020) COVID-19 among Healthcare Workers: Risk of Exposure, Impacts and Biosafety Measures–A Review. International Journal of Health, Safety and Environment 6: 534-548.
19. Corman V M, Landt O, Kaiser M, Molenkamp R, Meijer A, et al. (2020) Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 25: 2000045.
20. Chu DKW, Pan Y, Cheng SMS, Hui KPY, Krishnan P, et al. (2020) Molecular diagnosis of a novel coronavirus (2019-nCov) by real time RT-PCR. Euro. Surveill 25: 2000045.
21. Zhang N, Wang L, Deng X (2020) Recent advances in the detection of respiratory virus infection in humans. J. med. Virol 92: 408-417.
22. Tang Y-W, Schmitz JE, Persing DH, Stratton CW (2020) Laboratory diagnosis of COVID-19: current issues and challenges. J Clin Microbiol 58: 512-20.
23. Ai JW, Zhang Y, Zhang HC, Xu T, Zhang WH (2020) Era of molecular diagnosis for pathogen identification of unexplained pneumonia, lessons to be learned. Emerg Microbes Infect 9: 597-600.
24. Loeffelholz MJ, Tang YW (2020) Laboratory diagnosis of emerging human coronavirus infections-the state of the art. Emerg Microbes Infect 9: 747-756.
25. Nigeria Centre for Disease Control (NCDC). FAQs on COVID-19 Diagnostics. 2020. https://www.ncdc-FAQS-on-covid-19-diagnostics.
26. World Health Organization (WHO) (2020) Laboratory testing for coronavirus disease (COVID-19) in suspected human cases. 2020. https://www.who-laboratory-testing-for-coronavirus-disease-covid-19-in-suspected-human-cases.
27. Wong ML, Medrano JF (2005) Real-Time PCR for mRNA Quantitation. BioTechniques 39: 75-85.
28. Nigeria Centre for Disease Control (NCDC) (2020) COVID-19 Sample collection, packaging and transport. https://www.Covid-19-sample-collection-packaging-and-transport/
29. Wu F, Zhao S, Yu B, Chen Y-M, Wang W, et al. (2020) A new coronavirus associated with human respiratory disease in China. Nature 579: 265-269.
30. Lam TT-Y, Jia N, Zhang Y-W, Shum M H-H, Jiang J-F, et al. (2020) Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. Nature 583: 282-285.
31. Zou L, Ruan F, Huang M, Liang L, Huang H, et al. (2020) SARS-CoV-2 viral load in upper respiratory specimens of

infected patients. N Engl J Med 382: 1177-1179.
32. Druce J, Garcia K, Tran T, Papadakis G, Birch C (2012) Evaluation of swabs, transport media, and specimen transport conditions for optimal detection of viruses by PCR. J Clin Microbiol 50: 1064-1065.
33. International Standards Organization (ISO) Medical Laboratories: Requirements for Quality and Competence. ISO 15189:2012; 2014. https://www.iso.org/standard/56115.ht ml.
34. Wilfinger WW, Mackey K, Chomczynski P (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques 22: 474-¬481.
35. Fleige S, Pfaffl MW (2006) RNA integrity and the effect on the real-time qRT-PCR performance. Mol Aspects Med 27: 126-139.
36. Freeman WM, Walker SJ, Vrana KE (1999) Quantitative RT-PCR: pitfalls and potential' BioTechniques 26: 112-125.
37. Biovendor Research and Diagnostic Products. Liferiver Novel Coronavirus (2019-nCoV) Real time multiplex RT-PCR kit (detection of 3 genes) User manual. Revision No. ZJ0001, issue date: January 22, 2020. https://www.biovendor.com/liferiver-novel-coronavirus-2019-ncov-real-time-multiplex-rt-pcr-kit?utm_source=google&utm_medium=organic.
38. Broughton J P, Deng X, Yu G, Fasching CL, Servellita V, et al. (2020) CRISPR–Cas12-based detection of SARS-CoV-2. Nat Biotechnol 38: 870-874.
39. Sethuraman N, Stanleyr S, Ryo A (2020) Interpreting Diagnostic Tests for SARS-CoV-2. Journal of the American Medical Association, https://jamanetwork.com/.
40. Centers for Disease Control and Prevention (CDC) Coronavirus Disease-2019 (COVID-19): How to Report COVID-19 Laboratory Data. https://www.cdc.gov/coronavirus/2019-ncov/lab/reporting-lab-data.html
41. Nigeria Centre for Disease Control (NCDC) COVID-19: Laboratory Line List Reporting Form. Instructions for completing the COVID-19 laboratory line list. 2020 https://covid19.ncdc.gov.ng/guideline/
42. Lone S A, Ahmad A (2020) COVID-19 pandemic–an African perspective, Emerging Microbes & Infections 9: 300-1308.
43. World Health Organization (WHO) (2020) Critical Preparedness, Readiness and Response Actions for COVID-19: Interim Guidance, https://apps.who.int/iris/bitstream/handle/10665/331511/essreadinessandresponseactionsCOVID-102020-03-22_FINAL-eng.pdf.
44. Hasell J (2020) What can data on testing tell us about the pandemic? https://ourworldindata.org/what-can-data-on-testing-tell-us-about-the-pandemic.
45. Chan JF-W, Yip CC-Y, To KK-W, Tang T H-C, Wong SC-Y, et al. (2020) Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens. J Clin Microbiol 58: e00310-20.
46. Emorinken M (2020) Nigeria tests 6,000 COVID-19 samples daily, says NCDC. The Nation https://thenationonlineng.net/nigeria-tests-6000-covid-19-samples-daily-says-ncdc/
47. National Institute of Communicable Diseases (NICD) (2020) Latest Confirmed Cases of COVID-19 in South Africa: Data Testing. https://www.nicd.ac.za/latest-confirmed-cases-of-covid-19-in-south-africa-25-aug-2020/
48. Brown TS, Walensky RP (2020) Serosurveillance and the COVID-19 Epidemic in the US Undetected, Uncertain, and Out of Control. doi:10.1001/jama.2020.14017.
49. Adebisi YA, Oke GI, Ademola PS, Chinemelum IG, Ogunkola IO, et al. (2020) SARS-CoV-2 diagnostic testing in Africa: needs and challenges. Pan Africa Medical Journal 35: 4. DOI: 10.11604/pamj.2020.35.4.22703.
50. Financial Times: Universities tapped in drive to step up COVID-19 testing (Accessed: 4 April 2020).
51. Kazeem Y (2018) Nigeria has become the poverty capital of the world. https://qz.com/africa/1313380/nigerias-has-the-highest-rate-of-extreme-poverty-globally/
52. World Bank (2020) Nigeria - Poverty & Equity Data Portal-World Bank Group. .http://povertydata.worldbank.org/poverty/country/NGA.
53. Kobia F, Gitaka J (2020) COVID-19: Are Africa's diagnostic challenges blunting response effectiveness? AAS Open Res 3: 4. DOI: 10.12688/aasopenres.13061.1.
54. Paterlini M (2020) COVID-19: Over 300 Italian doctors and scientists call for more testing. British Medical Journal 368: m1274.
55. Kanmodi KK, Kanmodi PA (2020) COVID-19 Stigmatization: A devil and a deep blue sea. Population Medicine https://doi.org/10.18332/popmed/121098.
56. Singh S, Bhutani S, Fatima H (2020) "Surviving the stigma: lessons learnt for the prevention of COVID-19 stigma and its mental health impact", Mental Health and Social Inclusion. https://doi.org/10.1108/MHSI-05-2020-0030.
57. Onwuzoo A (2020) Nigeria can't beat COVID-19 with patients fleeing isolation centres. Punch Healthwise. https://healthwise.punchng.com/nigeria-cant-beat-covid-19-with-patients-fleeing-isolation-centres/
58. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q (2020) Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis 24: 30113-30114.
59. To KK, Tsang OT, Leung WS, Tam AR, Wu TC (2020) Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 23: 30196-30191.
60. Wolfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, et al. (2020) Virological assessment of hospitalized patients with COVID-2019. Nature https://doi.org/10.1038/s41586-020-2196-x.
61. Kim C, Ahmed JA, Eidex RB, Nyoka R, Waiboci LW, et al. (2011) Comparison of nasopharyngeal and oropharyngeal swabs for the diagnosis of eight respiratory viruses by real-time reverse transcription-PCR assays. PLoS One 6: e21610.
62. Li Y, Yao L, Li J, Chen L, Song Y, et al. (2020) Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19. J Med Virol https://doi.org/10.1002/jmv.25786.
63. Yu F, Yan L, Wang N, Yang S, Wang L, et al. (2020) Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients. Clin Infect Dis 71: 793-798.
64. NPR (2020) Why it takes so long to get most COVID-19 Test Results: Shots - Health News.
65. ECDC (2020) An overview of the rapid test situation for COVID-19 diagnosis in the EU/EEA. 10.1101/2020.03.18.20038059.
66. Udo B (2020) COVID-19: DisCos promise Nigerians free electricity for two months. Premium Times. https://www.premiumtimesng.com/news/headlines/386848-covid-19-discos-promise-nigerians-free-electricity-for-two-months.html
67. Olówósejéjé SA (2020) COVID-19: Nigeria should prioritize power supply to health care facilities. The Conservation https://theconversation.com/covid-19-nigeria-should-prioritise-power-supply-to-health-care-facilities-134444.
68. MacLeod J (2020) Petition: Fair wages and hazard pay for Laboratory Professionals during COVID-19 pandemic.

https://www.change.org/p/the-whitehouse-fair-wages-and-hazard-pay-for-laboratoryprofessionals-during-covid-19pandemic?source_location=topic_page.

69. Anadolu (2020) Agency. Nigeria: 113 healthcare workers infected with COVID-19. https://www.aa.com.tr/en/africa/nigeria-113healthcare-workers-infected-with-covid19/1825398
70. Bustin SA (2004) A-Z of Quantitative PCR; International University Line: San Diego, CA.
71. Jasper FC, Cyril CY, Kelvin KWT, Tommy HCT, Sally CYW, et al. (2020) Improved Molecular Diagnosis of COVID-19 by the Novel, Highly Sensitive and Specific COVID-19-RdRp/Hel Real-Time Reverse Transcription-PCR Assay Validated in vitro and with Clinical Specimens. Journal of Clinical Microbiology DOI: 10.1128/JCM.00310-20.
72. Tahamtan A, Ardebili A (2020) Real-time RT-PCR in COVID-19 detection: issues affecting the results. Expert review of molecular diagnosis 20: 453-454.
73. Phan T (2020) Genetic diversity and evolution of SARS-CoV-2. Infect Genet Evol81: 104-260.
74. Shen Z, Xiao Y, Kang L, Ma W, Shi L, et al. (2020) Genomic diversity of SARS-CoV-2 in coronavirus 2019 patients. Clin infect Dis 71: 713-720.
75. Yang P, Luyao L, Daitao Z, Tingting Y, Shujuan C, et al. (2020) Potential False-Negative Nucleic Acid Testing Results for Severe Acute Respiratory Syndrome Coronavirus 2 from Thermal Inactivation of Samples with Low Viral Loads, Clinical Chemistry 66: 794-801.
76. Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, et al. (2020) Washington State 2019-nCoV Case Investigation Team. First case of 2019 novel coronavirus in the United States. N Engl J Med 382:929 –936. https://doi.org/10.1056/NEJMoa2001191.
77. China National Health Commission (CNHC) (2020) New coronavirus pneumonia prevention and control protocol, 7th ed. National Commission of the People's Republic of China. (In Chinese.) http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989/files/ce3e6945832a438 eaae415350a8ce964.pdf
78. Chan KH, Poon LLLM, Cheng VCC, Guan Y, Hung IFN, et al. (2004) Detection of SARS Coronavirus in patients with suspected SARS. Emerg Infect Dis 10: 294-299.
79. Guo L, Ren L, Yang S, Xiao M, Chang D, et al. (2020) Profiling Early Humoral Response To Diagnose Novel Coronavirus Disease (COVID-19). Clin Infect Dis 71: 778-785.
80. Isakbaeva ET, Khetsuriani N, Beard RS, Peck A, Erdman D (2004) Monroe SS et al. SARS-associated coronavirus transmission, United States. Emerg Infect Dis 10: 225-23.
81. Nigeria Centre for Disease Control (NCDC) An Updated National COVID-19 Case Management Guidelines. https://ncdc.gov.ng/reports/261/2020-may-week-21.
82. Lohse PT, Berkó-Göttel B, Rissland J, Geißler T, Gärtner B, et al. (2020) Pooling of samples for testing for SARS-CoV-2 in asymptomatic people. The Lancet Infectious Diseases S1473-3099. doi.org/10.1016/S1473-3099(20)30362-5.
83. World Health Organization Africa (WHO-Africa) (2020) Pooling samples boosts Ghana's COVID-19 testing. WHO-Africa International News https://www.afro.who.int/news/pooling-samples-boosts-ghanas-covid-19-testing.