Coronavirus Pandemic

Roles and challenges of coordinated public health laboratory response against COVID-19 pandemic in Africa

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Abstract
As the incidence of Coronavirus Disease 19 (COVID-19) continues to rise, many countries have been seeking for medical assistance such as donation or procurement of laboratory test kits and strips. These consumables are largely intended for use in the laboratory investigations of COVID-19 cases, suspected contacts, asymptomatic persons and in discharging cured persons. Thus, this article was instigated to update and remind healthcare providers and policymakers (especially those in developing countries) on the principles of sample collections, storage, transportation, laboratory protocols and networks needed for appropriate public health response against COVID-19 pandemic in Africa and other developing countries. In addition, this article presents challenges that hinder adequate COVID-19 laboratory response and discuss some possible solutions that could ameliorate these constrains.

Key words: Molecular diagnostics; SARS-CoV-2; COVID-19; Laboratory tests.

Introduction

Based on recent available scientific data, the transmission, maintenance and expansion of severe acute respiratory syndrome coronavirus- 2 (SARS-CoV-2) infection revolve around humans, animals and the environment [1]. SARS-CoV-2, one of the three highly pathogenic coronaviruses and the etiological agent of Coronavirus Disease 19 (COVID-19) has thrown the world into pandemonium. As at 6:00AM GMT+1, 19th June 2020, there were 8,583,838 global confirmed cases of SARS-CoV-2 infection with a case fatality rate (CFR) of 5.3% (https://worldometers.info/coronavirus/)

Initially, the spread of SARS-CoV-2 in Africa was initially slow with few confirmed cases in Egypt after its first index case on 14th February. However, as of 18th June 2020, there were 191993 confirmed COVID-19 cases have been reported with a case fatality of 2.3% [2]. Several observers have attributed the low reported incidence rate of COVID-19 in most African countries to be due to under-diagnosis probably because of inadequate molecular diagnostic capacity (Table 1) and few manpower skilled in molecular diagnostic tests.

Considering the continuous rise in the CFR associated with COVID-19, there is a need for concerted efforts from multiple sectors targeting humans, putative animal hosts and the environment. To achieve this, it is believed that adoption of the "One Health" strategy will be an ideal public health intervention. This public health model is arguably a great fit in combating most infectious diseases.

For now, securing human health, minimizing COVID-19 associated morbidity and mortality are paramount to every country. Based on these, it is very essential for public health authorities to have coordinated case detection and contacts tracing system; and infection control measures. Indeed, all these can adequately be achieved through the availability and

implementation of specific, sensitive and accurate laboratory protocols for COVID-19 diagnostics. The decision to select and embark on laboratory test(s) should be based on the combination of clinical, epidemiological factors and likelihood of COVID-19 assessment [3]. As confirmed cases of COVID-19 continue to rise in all countries and territories, there are efforts in the production, evaluation and adoption of various COVID-19 diagnostic protocols.

Structural Organization of SARS-CoV-2

Virologically, SARS-CoV-2 is single stranded RNA virus with positive polarity and variable open reading frames (ORFs) [4]. It has been shown that two-third of SARS-CoV-2 genome are located within the 1st ORF which translates the pp1a and pp1ab polyproteins. These polyproteins encode 16 non-structural proteins [4]. Conversely, the remaining ORFs code viral structural and accessory proteins of SARS-CoV-2. The remaining one third genome codes the nucleocapsid (N) protein, spike (S) glycoprotein, matrix (M) protein, and small envelope (E) protein of SARS-CoV-2 [4]. Club-shaped glycoprotein projections are found on the envelop of the virion. Out of these 4 proteins, the S glycoprotein is key because of its role in host cells attachment and pathogenesis of COVID-19. This protein alongside the viral RNA dependent RNA Polymerase (RdRP) have largely been utilized in the synthesis of primers and antigens for molecular and serological tests of SARS-CoV-2 infection, respectively [5].

Clinical Laboratory Protocols for COVID-19

Diagnostically, the use of viral culture for establishing acute COVID-19 diagnosis is not practicable due to its long turnaround time (3 days) for SARS-CoV-2 to cause obvious cytopathic effects (CPE) on Vero E6 cells. In addition, isolation of SARS-CoV-2 is laborious and requires biosafety level-3 (BSL-3) facilities which are unavailable in most healthcare centers, especially in developing countries. So far, all available serum antigen (such as the S-glycoprotein) and antibody (IgA, IgM and IgG) detection tests have not been validated by the World health organization (WHO). However, it has been suggested that serological assays could assist in the analysis of an ongoing SARS-CoV-2 outbreak and retrospective evaluation of the incidence rate of an outbreak [6]. In some instances where epidemiological data of suspected cases correlates to SARS-CoV-2 infection, the demonstration of fourfold rising antibody titer between acute and convalescent phase sera could support diagnosis of COVID-19 when RT-PCR results are negative [7]. In addition, it has been revealed that a significant proportion of COVID-19 patients had tested RT-PCR negative despite having suitable clinical features and radiologic findings highly suspicious of SARS-CoV-2 infection [8]. In most cases, these are termed false negatives which could have been due to

Table 1. Top 20 COVID-19 most affected African Countries (As of 6 AM GMT+1 June 19th, 2020) [2].

Countries	Total Confirmed Cases	Mortality	Severe Cases	No. People Tested	National Population
South Africa	83,890	1,737	208	1,228,098	59,279,420
Egypt	50,437	1,938	41	135,000	102,252,449
Nigeria	18,480	475	7	106,006	205,906,213
Ghana	12,929	66	6	261,319	310,44,696
Algeria	11,385	211	54	NA	43,817,763
Cameroon	9,864	276	28	NA	26,515,641
Morocco	9,074	213	7	48,8947	36,893,608
Sudan	8,020	487	0	401	43,803,300
Ivory Coast	6,444	49	0	41,443	26,348,445
Senegal	5,475	76	16	63,862	16,723,391
Democratic Republic of Congo	5,283	117	0	NA	89,428,534
Guinea	4,841	26	24	14,407	13,116,064
Djibouti	4,557	43	0	43,871	987,423
Gabon	4,340	32	15	26,041	2,223,369
Kenya	4,257	117	7	130,498	53,718,978
Ethiopia	3,954	65	30	202,214	11,483,3537
Somalia	2,719	88	2	NA	15,872,205
Central African Republic	2,605	19	2	18,921	4,826,268
Mauritania	2,424	97	8	13,842	4,643,974
Mali	1909	107	NA	9873	20227492

NA: Not Available.

wrong sampling where SARS-CoV-2 might have been present in the lower respiratory tracts rather than upper respiratory samples often collected during laboratory diagnosis. Hence, this poses a challenge in the proper evaluation of SARS-CoV-2 symptomatic patients.

It has been reported that the kinetics of antibody response during SARS-CoV-2 infection shows anti-SARS-CoV-2 IgG which depends on the clinical stage of infection and viral load. In most IgM and IgG based Enzyme linked immunosorbent assays (ELISA), the coronavirus Rp3 nucleocapsid protein have been used as an antigen impregnated on the 96 well microtiter plates [7]. The kinetics of antibody response during SARS-CoV-2 infection shows anti-SARS-CoV-2 IgG serum levels rise when anti-SARS-CoV-2 IgM levels begin to decline at day 7 [9]. However, anti-SARS-CoV-2 IgM are only detectable by the third day after infection [7,8]. The technical rigor and quality of specimen requirement are relatively lesser in serological assays than Nucleic Acid Amplification Tests (NAATs).

Three major concerns with serological tests are the possibility of missing acute infection (by IgM) during the "window period" (i.e. 1st and 2nd days after infection); need for paired sampling (for IgG) at different intervals (acute and convalescence) and the possibility to cross- react with other SARS-CoVs, which share high degree (~80%) of nucleotide homology with SARS-CoV-2 [9]. Due to these limitations, molecular tests remain the most useful and gold standard laboratory protocol for COVID-19. However, to enable prompt and largescale testing with short test turnaround time, there is need for several alternative protocols that could augment RT-PCR. Currently, the antibody responses against SARS-CoV-2 has not been completely understood and the clinical utility of serological testing is under intensive evaluations.

Sample Collection Transportation and Laboratory Networks

During sample collection, its crucial to adhere to the standard infection control practice and the use of complete personal protection equipment. Because the SARS-CoV-2 is an enveloped virus (fragile), there is a need for utmost care in sample preparation and its transportation. Samples dispatched to molecular virology laboratory need to be perseved and transported in viral transport medium (VTM) containing antifungal and antibiotic supplements at -70°C (dry ice) to +8°C depending on lag time before laboratory analysis [10].

To enable prompt and short test turnaround time, there is a need for several functional molecular laboratory networks and adequate laboratory specialists who are proficient in hands-on molecular diagnostic techniques. These should ideally be available in ≤ 100 kilometers between two locations, for instance, one accredited testing site between two nearby cities. In the absence of proximal laboratories, countries could consider adopting mobile laboratories vans for sample collection and transportation to regional laboratories.

Current Diagnostic Criteria for COVID-19

The availability of complete genome sequences of SARS-CoV-2 during the early period of COVID-19 epidemic facilitated the development of specific primers and standardized laboratory protocols for SARS-CoV-2. SARS-CoV-2 RNA can be detected within 1-2 days from upper respiratory tract samples before the onset of clinical symptoms [11]. There are reports of the persistence of the viral RNA in moderate cases for 7-12 days and even up to 14 days in severe cases [11]. In fact, there has been reports of prolonged SARS-CoV-2 shedding from nasopharyngeal fluids up to 24 days post onset of symptoms [12]. However, this doesn't necessarily indicate infectiousness of the patients, probably due to the non-viability of SARS-CoV-2 in such patients, as their samples did not yield cytopathic effects on Vero E6 cell lines [13]. From day 5 post onset of symptoms, viral RNA has also been detected from stool of 30% of COVID-19 patients and this persisted for 4-5 weeks in moderate COVID-19 cases. However, the public health significance of fecal SARS-CoV-2 shedding is yet to be confirmed [3]. One important thing to consider during test selection is possibilities of false positive and negative results due to contaminations, poor and inadequate sampling.

Clinically, sustained virologic clearance of SARS-CoV-2 is defined after at least 2 sets of samples each from lower and upper respiratory tracts collected at > 24-hour intervals turn out negative by RT-PCR, > 7 days post onset of symptoms, or > 3 days after febrile symptoms [14]. For persons with asymptomatic SARS-CoV-2-infection, a minimum of 14 days is required to be observed after the initial positive test before taking the test to document the virus clearance [14]. It is important to use RT-PCR targeting two different genes at different occasions e.g. E-gene and RdRP, for confirmation and discharging patients as negative. It is very important to include internal quality control and external quality assurance measures of all test run and test kits batches, respectively to ensure precision and reproducibility of tests results.

Roles of Laboratory-based Surveillance during COVID-19 Pandemic

To achieve adequate COVID-19 monitoring and surveillance, there is need for widespread and continuous testing of not only suspected cases or contacts, but even the asymptomatic and apparently healthy populations. This is to meet with recommendation of the WHO of comprehensive public health response against COVID-19 [15]. This include recommendation aimed to achieve adequate COVID-19 trend monitoring in contexts of rapid human-to-human spread, prompt identification of cases in locations where SARS-CoV-2 was previously not in circulation, risk assessment through good epidemiological data from different geographical levels and provide epidemiological data which will direct rational government policies for adequate response and resource distribution. However, the extent at which this laboratory-based surveillance could be implemented depends on country's prevailing peculiarity and choices for COVID-19 control and prevention measures.

Major Challenges associated with COVID-19 Diagnostics in Africa

Undoubtedly, there are many benefits of large-scale population testing for COVID-19, as demonstrated in many high-income countries. But this cannot be achievable in most of African countries, due to their inadequate capacity for large scale laboratory testing. Before the emergence of SARS-CoV-2, most Africa countries had numerous challenges in their healthcare delivery system, especially in terms of laboratory diagnostics, house-to-house case searching and community contact tracing for infectious diseases surveillance. Typified by the inadequate laboratory testing capacity, Nigeria being the most populous African Nation with over 206 million inhabitants was only able to test some 106,006 persons across its 30 testing sites, as of 19th June 2020 [16]. This is mainly due to inadequate test kit and skilled laboratory personnel due to high demand for COVID-19 tests. The major testing challenge was due the adoption and nature of RTPCR as gold standard for COVID-19 diagnosis. These molecular assays kits, equipment not cost effective, making it difficult for most African countries to procure.

Aside from technical difficulties associated with COVID-19 testing, certain seasonal changes might equally affect the number of people tested. For instance, enrolling people from remote villages and densely populated slums and communities during the raining season will pose a great hurdle. The rainy season is about to or has started in most part of Africa. This will make many roads stumpy, dirty and impassable, hence rendering many rural communities inaccessible for adequate case tracing and testing. In addition, slums and overcrowded location but are mostly not mapped and inaccessible. Hence, administering systematic large-scale testing in such settlements will be very challenging, especially during the rainy season. In this situation, mobile laboratories vans and bikes men for sample collection will help minimize this constrain.

Possible Solutions and Alternatives for COVID-19 Diagnostic Challenges

As the incidence and fatality rates of COVID-19 continue to rise in all countries, there are ongoing efforts geared to provide alternative COVID-19 diagnostic protocols aside from the RTPCR. Due to the impact of COVID-19 on the global economy, there might be scarcity of COVID-19 test kits and reagents. Hence, there is a need for countries to urgently "think out of the box" and consider evaluation and validation of SARS-CoV-2 ELISA and rapid test strips, in order to scale up the test capacity of the public health laboratory system. Due to the consequences of inadequate largescale testing capacity in Africa and beyond, many public institutions and private firms have developed and are evaluating rapid test kits with the hope of reducing the test turnaround time and expand the test capacity in order to get better epidemiological data of SARS-CoV-2 spread. Most of these immunoassays can directly detect SARS-CoV-2 structural proteins or indirectly detect anti-SARS-CoV-2 [17]. Some of these promising SARS-CoV-2 test protocols are the 96 well Enzyme Linked Immunosorbent Assay (ELISA) and lateral flow immunochromatography rapid diagnostic test (RDT). So far, all available serum antigen and antibody (IgA, IgM and IgG) detection tests have not been validated by the WHO. However, it has been suggested that serological assays could assist in the analysis of an ongoing SARS-CoV-2 outbreak, retrospective evaluation of the incidence rate of an outbreak and could support diagnosis of COVID-19 when RT-PCR results are negative [4]. In addition, RDTs for both IgM and IgG antibodies undoubtedly will play an important role in the detection of asymptomatic cases and determine the immunity of health care workers as the outbreak progresses [18].

Conclusions

As the incidence rate of COVID-19 continue to rise, it is crucial for all nations to make available sufficient laboratory testing services and standardized testing kits.

Considering the current scarcity of medical and diagnostic consumables and equipment, it is necessary for scientists to consider proper adoption and evaluation of commercially produced test kits to scale up testing capacity *viz a viz* avoid inaccurate results. In addition, it is recommended to consider local production and standardization of SARS-CoV-2 primers preferable using sequences from local strains to augment the currently available and certified test protocols.

References

1. Rodriguez-Morales AJ, Bonilla-Aldana DK, Balbin-Ramon GJ, Rabaan AA, Sah R, Paniz-Mondolfi A, Pagliano P, Esposito S (2020) History is repeating itself: Probable zoonotic spillover as the cause of the 2019 novel Coronavirus Epidemic. Infez Med 28: 3-5.
2. African Center for Disease Control (2020) Coronavirus Disease 2019 (COVID-19) in Africa. Available: https://africacdc.org/covid-19/ Accessed: 19th June, 2020
3. World Health Organization (2020) Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID19). Available: https://www.who.int/docs/default-source/coronaviruse/whochina-joint-mission-on-covid-19-final-report.pdf.; Accessed: 4th April, 2020.
4. Cui J, Li F, Shi ZL (2019) Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 17: 181–192.
5. World Health Organization (2020) Global surveillance for human infection with coronavirus disease (COVID-2019), Interim guidance, Geneva. Available: https://www.who.int/publicationsdetail/global-surveillance-for-humaninfection-with-novel-coronavirus-(2019-ncov). Accessed: 4th April, 2020.
6. World Health Organization (2020) Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases. Available: https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117. Accessed: 12th April, 2020.
7. Xiao S, Wu Y, Liu H (2020) Evolving status of the 2019 novel coronavirus infection: Proposal of conventional serologic assays for disease diagnosis and infection monitoring. J Med Virol 92: 464–467.
8. Zhou P, Yang XL, Wang XG (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579: 270-273.
9. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, Tan KS, Wang DY, Yan Y (2020) The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. Mil Med Res 7: 1-10.
10. World Health Organization (2019) Guidance on regulations for the transport of infectious substances 2019–2020. Geneva. Available: https://www.who.int/ihr/publications/WHO-WHE-CPI-2019.20/en/. Accessed: 4th April, 2020.
11. Chan JFW, Yip CCY, To KKW, Tang THC, Wong SCY, Leung KH, Fung AYF, Ng ACK, Zou Z, Tsoi HW, Choi GKY, Tam AR, Cheng VCC, Chan KH, Tsang OTY, Yuen KY (2020) Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific 2 COVID -19 -RdRp/Hel real -time reverse transcription -polymerase chain reaction assay validated 3 *in vitro* and with clinical specimens. J Clin Microbiol 58: e00310-320.
12. Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J (2020) Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA 323(15):1488-1494.
13. Hoehl S, Rabenau H, Berger A, Kortenbusch M, Cinatl J, Bojkova D, Behrens P, Böddinghaus B, Götsch U, Naujoks F, Neumann P, Schork J, Tiarks-Jungk P, Walczok A, Eickmann M, Vehreschild MJGT, Kann G, Wolf T, Gottschalk R, Ciesek S (2020) Evidence of SARS-CoV-2 infection in returning travelers from Wuhan, China. New Eng J Med 382: 1278-1280.
14. European Centre for Disease Prevention and Control (ECDC) (2020) Novel coronavirus (SARS-CoV-2). Discharge criteria for confirmed COVID-19 cases - When is it safe to discharge COVID-19 cases from the hospital or end home isolation?. Available: https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-Discharge-criteria.pdf. Accessed: 4th April 2020.
15. World Health Organization (2020) Global Surveillance for human infection with coronavirus disease(COVID-19). Interim Guidance. Available. https://www.who.int/docs/default-source/coronaviruse/global-surveillance-for-covid-v-19-final200321-rev.pdf. Accessed: 24th April, 2020.
16. Nigerian Center for Disease Control and Prevention (2020) COVID-19 situation report situation Report. Available: https://ncdc.gov.ng/themes/common/files/sitreps/c7c73f429bbda476f1419011e1a10403.pdf. Accessed: 20th June, 2020.
17. European Center for Disease Control (2020) An overview of the rapid test situation for COVID-19 diagnosis in the EU/EEA. 2020. Available: https://www.ecdc.europa.eu/en/publications-data/overview-rapid-test-situation-covid-19-diagnosis-eueea. Accessed: 10th May, 2020.
18. Abdullahi IN, Emeribe AU, Mustapha JO, Fasogbon SA, Ofor IB, Opeyemi IS, Obi-George C, Sunday AO, Nwofe J (2020) Exploring the genetics, ecology of SARS-COV-2 and climatic factors as possible control strategies against COVID-19. Infez Med 28: 166-173.

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