Rapid, Cheap, and Effective COVID-19 Diagnostics for Africa

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Contributor: Lukman Yusuf

In Africa and other low- and middle-income countries there is high rate of COVID-19 under-diagnosis, due to the high cost of molecular assays. Exploring alternate assays to the reverse transcriptase polymerase chain reaction (RT-PCR) for COVID-19 diagnosis is highly warranted.

Keywords: COVID-19 diagnostics ; LAMP

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is highly infectious, akin to its predecessors SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), which triggered epidemics in 2003 and an ongoing one since 2012, respectively [1]. The transmission of SARS-CoV-2 in Africa was initially slow, with few reported cases in Egypt following its index occurrence on 14 February 2020 [2]. In most African countries, many observers attributed the low recorded incidence rate of COVID-19 to under-diagnosis [2]. The disease had already spread rapidly across the world, with the World Health Organization (WHO) declaring Coronavirus disease 2019 (COVID-19) a pandemic on 24 February 2020. While globally, public health initiatives such as mass quarantine that have been instituted have been ineffective, the incidence of COVID-19 increased in the early days of the COVID pandemic with a high infection rate. The rapid spread of SARS-CoV-2 requires effective control in every part of the world with early case detection to halt transmission. This effective control of SARS-CoV-2, like other viruses, relies on finding and developing robust therapeutics, as well as simple, effective, and rapid diagnostics [3]. There is therefore an immediate global need to increase the diagnostic capacity everywhere. However, there is a global shortage of PCR reagents and swabs, as well as reports of discordant results from different COVID-19 tests. This could be due to the differences in the cycle threshold (Ct) cut-offs being used. Studies have analyzed the association between Cycle threshold (Ct) levels and the possibility of growing a live virus. It was reported that the Ct was much lower and log copies were significantly greater in those with live viral cultures, according to the findings. Other studies using Ct cut-off values ranging from Ct > 24 to Ct > 35 found no growth in the specimens. The likelihood of recovering the virus from specimens with Ct > 35 was calculated to be 8.3% (95% CI: 2.8% to 18.4%). Based on this, some kits/machines have lower cut-offs and other more stringent ones have higher Ct cut-offs. A way to address this is by repeated sampling over a few days—an expensive process by PCR. Therefore, the need for fast rapid diagnoses that aid in clinical decision-making and the need to consider alternate testing methods make the development of new options necessary.

2. Laboratory Diagnosis of SARS-CoV-2

During the early COVID-19 epidemic era, the availability of SARS-CoV-2 genome sequences, which were made available since 10 January 2020, facilitated the production of unique primers and standard laboratory protocols for the diagnosis of the virus [2]. The World Health Organization (WHO) subsequently recommended the use of quantitative reverse transcription polymerase chain reaction (qRT-PCR) for nucleic acid amplification as the gold standard for the diagnosis of active SARS-CoV-2 infection [4]. Under this recommendation, suspected shedders/cases, as well as asymptomatic and mildly symptomatic shedders/cases, were all to be confirmed by RT-PCR. Most current COVID-19 RT-qPCR-based tests target the ORF1ab region of the SARS-CoV-2 genome, in addition to the coding sequences of either the E or N proteins [5].

SARS-CoV-2 RNA can be identified from upper respiratory tract samples within 1–2 days before clinical symptoms occur. In mild cases, the persistence of the viral RNA has been recorded for 7–12 days. Virus shedding can last for up to 14 days in extreme cases. There have even been reports of sustained shedding of SARS-CoV-2 from nasopharyngeal fluids up to 24 days after symptom onset [2]. This does not, however, necessarily suggest patient's infectiousness, possibly because

of the non-viability of SARS-CoV-2 in such patients, as their specimens did not produce CPE on Vero E6 cell lines. The chances of false positive and negative results due to contamination, as well as bad and insufficient sampling, are an essential factor to consider during the selection of test methods. Nonetheless, although some molecular diagnostic kits for SARS-CoV-2 detection have been marketed, real-time RT PCR is still the most accepted choice of test.

3. Limitations of RT-PCR Testing

RT-PCR offers highly precise and sometimes quantitative SARS-CoV-2 RNA detection. It is, however, complicated, costly, and slow to execute. A single RT-PCR test kit can cost more than 100 US dollars, and it takes more than 15,000 US dollars to set up a diagnostic laboratory [6]. The analysis time of the RT-PCR available in most African countries is not less than 4 h, as depicted in **Table 1**, while the turn-around period is more than 24 h, from the sample collection to the readiness of the result. Although the Xpert Xpress SARS-CoV-2 test is a rapid automated in vitro diagnostic test that detects nucleic acid of SARS-CoV-2 with very short turn-around-time, it requires the use of expensive cartridges and/or machines, an example of which is the GeneXpert instrument system, and are expensive and require expertise.

Table 1. Some of the available SARS-CoV-2 diagnostic tests.

Test	Strength	Limitations	Time of Analysis	Cost (USD)
RT-PCR	**High sensitivity and specificity**	**Requires expertise**	**4 h**	**300–6700**
LAMP	**High sensitivity and specificity, and a shorter turnaround time**	**Requires expertise**	**25 min**	**230–1550**
Rcombinase Polymerase Amplification	**High sensitivity and specificity, and a shorter turnaround time**	**Requires expertise**	**20 min**	**270**
Antigen-RDT	**Faster turnaround time and does not require expertise**	**Lower sensitivity**	**15 min**	**3–75**
Antibody-RDT	**Faster turnaround time and does not require expertise**	**Lower sensitivity**	**15 min**	**2.50–75**

In addition, molecular diagnostics are not accessible for many developing and underdeveloped countries due to their cost and the need for a trained clinical laboratory professionals and laboratories with a high complexity to operate. Furthermore, some studies have indicated a high discordant result rate for SARS-CoV-2 RT-PCR diagnostics, which may be due to inappropriate sample collection, purification, processing, and Ct, especially during the pandemic era [7]. Other factors can also cause false negative results, such as purified RNA degradation, the existence of RT-PCR inhibitors, or genomic mutations; some of these limitations have made the requirement for serology-based tests a necessity.

4. Constraints to COVID-19 Mass Testing by RT-PCR

4.1. Finance and Infrastructure

There are numerous advantages to massive population testing for COVID-19, as many high-income nations have shown. According to Liang et al. (2020), a higher COVID-19 mortality rate can be linked to fewer tests. However, most African countries are unable to achieve mass testing due to the shortage of large-scale laboratory testing capacities [2]. Most African countries faced many difficulties in their healthcare services prior to the advent of SARS-CoV-2, especially in terms of laboratory diagnostics, house-to-house case tracking, and community contact tracing for epidemiology. Nigeria, which is the most populous African country with over 206 million people, was only able to test 106,006 people across its 30 testing sites as of 19 June 2020, illustrating the lack of a laboratory testing capacity. This is because of the lack of test kits and qualified laboratory personnel as a result of the high demand for COVID-19 tests [2]. The acceptance and complexity of RT- PCR as the gold standard for COVID-19 diagnosis posed a significant testing impediment. This is due to the fact that this molecular assay's equipment and kits are not cost effective, making them expensive to obtain in several African countries.

4.2. Turn-Around-Time for Report

Another significant limiting factor in PCR testing is the duration of the diagnosis, which is a limiting factor for achieving the required mass testing. Aside from the technological challenges of COVID-19 screening, seasonal variations could have an effect on the number of people tested. For example, enrolling people from rural villages and heavily populated

shantytowns and communities would pose accessibility challenges during the rainy season [8][9]. In this circumstance, alternate quick and cheaper molecular tests and serological tests can be of immense help.

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