The implementation of antibody Rapid Diagnostic Testing vs. rRT-PCR sample pooling in the screening of COVID-19: A case of different testing strategies in Africa


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Abstract
COVID-19 has wreaked havoc across the globe, although cases in Africa remain lower than other regions but they are gradually on an upward trajectory. To date, COVID-19 cases have been reported in 54 countries. However, due to limited SARS-COV-2 rRT-PCR testing capacity and scarcity of testing reagents, it is probable that the total number of cases could far exceed published statistics. In this viewpoint, using Ghana, Malawi, South Africa and Zimbabwe as examples of countries that have implemented different testing strategies, we argue that the implementation of sample pooling for rRT-PCR over antibody rapid diagnostic testing could have a greater impact in assessing disease burden. Sample pooling offers huge advantages compared to single test rRT-PCR, as it lowers experimental costs, personnel time, reduces burnout and analytical run-times. Africa is already strained in terms of testing resources for COVID-19, hence cheaper alternative ways need to be implemented to conserve resources, maximise on mass testing and reduce transmission in the wider population.

Keywords
Antibody rapid diagnostic test, sample pooling, rRT-PCR, COVID-19, Africa
Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak was first reported in Wuhan, China, in December 2019, and has spread throughout the world with an unprecedented impact on humanity (1). The disease later termed coronavirus disease 2019 (COVID-19), has now been reported to have affected more than 6.2 million people worldwide culminating in over 376,000 deaths, by the 2nd June 2020 (2). In Africa, COVID-19 has been reported in 54 countries, with a cumulative 155,610 confirmed cases and 4,429 deaths (3). With over 3 billion people under COVID-19 induced lockdown worldwide, accompanied by the attendant negative impact on global economies, governments are racing to flatten the epidemic curve of COVID-19 in both space and time (4, 5). Some of the policies imposed by governments to flatten the curve include; travel bans, lockdowns, quarantining and widespread mass testing using real time reverse transcription polymerase chain reaction (rRT-PCR) (4, 5).

In order to achieve gains from these implemented measures and to reduce the burden of COVID-19 cases on societies and economies, it is critical to implement diagnostic strategies with high analytical sensitivity and specificity to enable early detection of SARS-CoV-2 cases for isolation, contact tracing, care and management. International collaborative efforts initiated after the emergence of the SARS-CoV-2 pandemic, led to the development of rRT-PCR diagnostic assays to support case ascertainment and outbreak tracking (5, 6). The rRT-PCR is reported to have a diagnostic sensitivity of 88 – 99% and a diagnostic specificity of 77 – 100% (7). This development thus, accelerated the definite diagnosis of asymptomatic, pre-symptomatic and symptomatic cases (6).
Due to limited SARS-CoV-2 rRT-PCR testing capacity and scarcity of testing reagents in many geographical regions including Africa, it is clear that the total number of COVID-19 cases is probably much higher than published confirmed statistics (8). Using Ghana, Malawi, South Africa and Zimbabwe as examples of countries that have implemented different testing strategies, we argue that the implementation of sample pooling for rRT-PCR over antibody rapid diagnostic testing (RDT) could have a greater impact in the early detection of the active COVID-19 cases, and assist in curbing the spread of the disease, whereas antibody based RDTs may help to ascertain community seroprevalence and estimate potential herd immunity benefits.

Case studies of COVID-19 diagnostic strategies in Africa

A case study of Ghana

Ghana is one of the few countries that implemented sample pooling strategy for COVID-19 rRT-PCR testing and confirmation (9, 10). However, at the onset of the pandemic, the country implemented single sample rRT-PCR testing before scaling it upwards through sample pooling (10, 11). As of 31st May 2020, a cumulative 219,825 tests had been conducted by the seven government affiliated laboratories spread across Ghana (12). Through sample pooling approximately ~0.71% of the total population have been tested, giving rise to 8,297 confirmed COVID-19 cases (positivity rate 3.77%) (12). Ghana is the 2nd highest ranked African country in terms of testing capacity. The COVID-19 testing strategy implemented, pools, at most 10 different patient samples for amplification prior to testing. This strategy has tremendously improved Ghana’s COVID-19 testing capacity and definitive diagnosis.
A case study of Malawi

As of 1st June 2020, Malawi had conducted 5,505 rRT-PCR tests yielding 358 COVID-19 cases (13). Malawi has the highest SARS-CoV-2 positivity rate (6.50%) in the region, despite its low testing rate of only ~0.03% of the population tested so far (13). The country recently increased its COVID-19 testing sites to 14, in order to expand geographic coverage. Owing to the low testing capacity, Malawi needs to be more restrictive in choosing who to test, in order to maximise benefits from its limited resources.

A case study of South Africa

South Africa has conducted 742,742 rRT-PCR tests yielding 35,812 COVID-19 cases and a positivity rate of 4.82% (14). It is the leading African country in terms of testing capacity, with 1.25% of its population having been tested for COVID-19 by the 1st June 2020. South Africa is currently conducting individual sample testing using several in-house and commercial PCR assays to test for the presence of SARS-CoV-2 RNA (15). However, most diagnostic laboratories are overwhelmed as the country is conducting targeted community symptom screening and testing for both symptomatic and asymptomatic individuals in some provinces (15, 16). Novel screening and testing strategies need to be adopted in order to manage and improve the capacity and sample turnaround testing time in diagnostic laboratories.

A case study of Zimbabwe

Recently, the Ministry of Health and Child Care (MoHCC) of Zimbabwe, introduced antibody RDT as a screening test strategy for SARS-CoV-2, to complement the rRT-PCR assays being offered in government reference laboratories, state universities and private laboratories.
Despite this development, the country still has limited capacity to conduct mass testing (17).

As of 1\textsuperscript{st} June 2020, a cumulative 46,021 COVID-19 tests (28,112 RDTs and 18,709 RT-PCR) had been conducted, with 203 confirmed cases (18). The current testing coverage translates to approximately ~0.13% of the population tested by rRT-PCR/ Xpert\textsuperscript{®} Xpress for SARS-CoV-2 with a 1.09% positivity rate. Furthermore, many of the RDTs in current use have not been validated or verified for use within this population. Thus, the current rRT-PCR under-testing raises the concern that many infected individuals are probably going undiagnosed.

**Implementation of different testing strategies for COVID-19**

**Sample pooling for rRT-PCR**

This technique was first suggested by Robert Dorfman in 1943 as a way of optimising syphilis testing, and since then, has been applied in surveillances and screening for infections such as human immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), malaria and influenza virus (19–25). Sample pooling has popularly been employed in serological work but also in molecular diagnostics and research. It helps to lower experimental costs, personnel time and reduces analytical run-times whilst maximising the level of surveillance and accuracy of the diagnostic test (19, 21, 24, 26). In public health emergency responses, such as the COVID-19 pandemic, rapidly growing workloads can outstrip laboratory testing capacity, resulting in reagent shortages and personnel burnout (16, 27). Thus, in large populations with a low disease prevalence (<10%), sample pooling provides a cost-effective strategy for screening asymptomatic and pre-symptomatic individuals (25, 27–29). Two different sample pooling strategies can be used namely; single-step pools, where prevalence and confidence intervals are calculated based on pool results (30), or multiple-step pools, where individual samples from a positive pool
are retested to identify the number of positive individuals per pool (31, 32). The latter being more useful for COVID-19 as the individuals need to be identified for isolation, contact tracing, care and treatment.

The use of a single test to screen groups of more than two people though labour intensive, provides a comprehensive, rapid and localised mass testing strategy required to identify cases acutely and minimise spread of disease. However, the anticipated cost reduction only becomes meaningful if statistical equivalence between the pooled and non-pooled experimental setups is achieved and maintained (33). In vitro and in silico studies of pooling nasopharyngeal samples for rRT-PCR assays, have reported the optimal pool size \( p \), to be five (5) samples per pool (28, 29). On account of variances in different PCR assay limits-of-detection (LoD), it is paramount to validate the use of pooled samples and rule out false negatives as a result of sample dilution. Of note, \( p \) can be up-scaled to \( p=32 \) (29, 34–36), where concentration of RNA by elution/lyophilisation becomes necessary to curb the risk of false negatives due to sample dilution (37). Fundamental principles for successful application of sample pooling are subject to the assay’s sensitivity and specificity, limit-of-detection and the prevalence of disease in the population.

COVID-19 is estimated to have a high effective reproductive number \( R_0 \) (ranging from 1.4 to 6.49, with a median 2.79) (38). Employing sample pooling for contact tracing ensures swift case identification, with less resources used to arrest community transmissions (38–40). Meanwhile, for frontline healthcare workers (HCW) interfacing with COVID-19 patients, sample pooling can be modelled to cluster high-risk individuals, in order to concentrate positivity rate to a few pools thereby further conserving resources.
Sample pooling is also associated with limitations such as; reduced test sensitivity in settings with very low infection prevalence (21, 41). Thus, a diagnostic laboratory has to optimise the pool size based on the prevalence of the infection within the society. Additionally, it is also critical for laboratories to understand that a negative pool result, would not distinguish between a true negative and an indeterminate or inconclusive result due to poor specimen collection or handling (24).

**Antibody RDTs testing for COVID-19**

Any acceptable screening or first line test should ideally have a high clinical diagnostic sensitivity, whereas the consecutive confirmatory test needs to have a high specificity (5, 7, 8, 42, 43). Despite this, some low-medium income countries (LMICs) in Africa have implemented unverified RDTs for COVID-19 into their testing and surveillance protocols. Whether these RDTs are yielding intended results remains to be verified. However, cheaper but well validated serological assays are essential to complement the fairly expensive rRT-PCR diagnostic assays to accurately assess the COVID-19 disease burden within communities and mapping global exposure (6). Samples can also be pooled as is applied when screening blood products for such infections as HBV and HCV to achieve maximum testing benefit (44).

Testing of specific antibodies against SARS-CoV-2 in patient blood is a good choice for rapid and simple diagnosis of COVID-19 (45). However, early studies have reported that the majority of COVID-19 patients seroconvert between days 7 and 11 post-exposure to the SARS-CoV-2 thus rendering antibody testing questionable in the setting of an acute illness.
Moreover, RDTs kits for SARS-CoV-2 antibodies have an analytical sensitivity of 69 – 88% for IgM and 90 – 99% for IgG (7, 8, 42, 45). Therefore, RDTs pose a challenge of; When to test? Whom to test? What to test? How often to test? and, what to do with test results? (43). Although validated RDTs can be used in sero-prevalence studies, data generated from use of RDT’s may not be reliable unless conducted systematically and periodically due to the wide variation in duration of time to seroconversion among individuals (16).

Scenarios where rRT-PCR sample pooling or antibody RDT may be employed.

Establishing the status of an asymptomatic individual.

COVID-19 follows an asymptomatic disease course before symptoms appear thus, causing increased spread of the disease (48, 49). Since antibodies can only be detected 6-29 days after symptom onset, antibody RDT’s may result in false negative COVID-19 status which will in turn delay management and contact tracing thus allowing propagation of infections within communities. On the contrary, various studies using rRT-PCR, have demonstrated asymptomatic carriage in obstetric patients (13.7%), Diamond Princess ship passengers (17.9%), skilled nursing facility residents (35.6%) (48–50). It should, however, be stressed that in the highlighted studies, the asymptomatic carriers contributed ~50% of the COVID-19 cases.

Testing Healthcare workers and other employees providing essential services.

Healthcare workers (HCW) and essential service employees showing no symptoms but continuously interfacing with the general population have been shown to be capable of transmitting SARS-CoV-2, as demonstrated in the skilled nursing facility residents in New
Mass testing for asymptomatic HCWs and employees in the essential services is therefore critical in order to mitigate workforce depletion by unnecessary quarantine; reduce spread of atypical, mild, or asymptomatic cases; and protect the healthcare and essential service workforce (51). Thus, it is crucial to set a reasonable testing schedule and frequency using pooled sampling rRT-PCR after assessing their risk profile to allow early detection and intervention in asymptomatic and pre-symptomatic individuals. This can be done by splitting employees working in the same department into groups and staggering testing of these groups to help identify any potential circulation (thus need of contact tracing) of the disease among staff while minimising once off use of resources.

Asymptomatic and pre-symptomatic HCWs and essential service workers are an underappreciated potential source of infection and worthy of testing to reduce in-hospital transmission and community spread (51, 52). Workers returning to work may be tested by validated RDTs as a means of tracing possible missed asymptomatic/presymptomatic and symptomatic cases. Furthermore, routine temperature checks must be conducted daily, collectively, this will aid in reducing community spread.

**Contact tracing**

Elucidation of the chain of infection and identification of the source of COVID-19 infections are crucial for effective disease containment (52, 53). Although the rRT-PCR option offers a diagnostic solution and is important for establishing infection status in contacts of an index case, this approach might not be diagnostically useful in recovered patients who are no longer shedding the virus (53). The duration of viral shedding for COVID-19 remains uncertain (54), but data from SARS-CoV indicate that 21 days after symptom onset, 53% of
cases achieved viral clearance in nasopharyngeal aspirate samples (55). Thus, serological tests are more useful in identifying convalescent cases, ascertaining seroprevalence and an accurate denominator for case fatality rate (53).

Establishing status in symptomatic patients

It is critical to establish the status of any person exhibiting COVID-19 related symptoms as soon as possible to enable appropriate management. As previously emphasised, RDTs have a limitation as far as detection at early onset is concerned thus, may not be very useful.

Establishing past exposure and immunity to COVID-19

Detecting individuals with past exposure or those that would have recovered from the SARS-CoV-2, by testing for IgM/IgG exposure is critical. However, whether the immune response following exposure to SARS-CoV-2 is long-lasting and protective against reinfection remains an issue of debate (5, 47, 56). Furthermore, laboratory tests that detect antibodies to SARS-CoV-2, including rapid immunodiagnostic tests, need further validation to determine their clinical utility (57).

Conclusions

The use of sample pooling for rRT-PCR testing particularly in Africa, to screen for active COVID-19 cases has a great advantage over single test rRT-PCR, as it helps lowering experimental costs, personnel time, burnout and also reduces analytical run-times (27, 29, 37, 48, 58). Africa is already strained in terms of testing resources for COVID-19, hence cheaper alternatives need to be implemented to conserve resources, maximise on mass testing and reduce transmission in the wider population. Currently, WHO does not
recommend the use of antibody and antigen-detecting rapid diagnostic tests for patient care but encourages the continuation of work to establish their usefulness in disease surveillance and epidemiologic research (57, 59). Healthcare workers and other essential services workers, particularly those working in cities and towns with confirmed cases are a key reservoir for the transmission of COVID-19, due to their interface with patients and the wider population, respectively. Thus, it is crucial to set a reasonable testing schedule and frequency using pooling of samples for rRT-PCR after assessing their risk profile to allow early detection and intervention in asymptomatic and pre-symptomatic individuals. Blanket testing of asymptomatic frontline staff is a futile exercise that will not add value to this fight.

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We declare no competing interests.
Key terms and definitions

Asymptomatic - laboratory-confirmed COVID-19 case who does not develop symptoms.

Pre-symptomatic – an individual who has been exposed to the virus (becoming infected) but hasn't developed symptom yet.

Seroconversion – It is the transition from a seronegative condition—where no antibodies are detectable in the serum or are present at titres below the limit of detection— to a seropositive condition, in which antibodies are detectable in serum samples.

Symptomatic - a case who has developed signs and symptoms compatible with COVID-19 virus infection

Sensitivity – the ability of a diagnostic test to correctly identify all patients with the disease.

Specificity – the ability of a diagnostic test to correctly identify all patients who do not have a disease.
References:


antibody responses during infection by SARS-CoV-2: an observational cohort study.


