Africa Centres for Disease Control and Prevention (Africa CDC)

Guidance on pooled testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

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Purpose of this document

The purpose of this document is to provide guidance on the use of pooled sample testing strategy in coronavirus disease (COVID-19) testing laboratories of the African Union Member States for scaling up SARS-CoV-2 nucleic acid testing capacity with the available resources. The current document describes the effect of factors such as the prevalence of COVID-19 in the population to be tested, the homogeneity of pools, and the sensitivity of the molecular test in optimal pool size determination. It also highlights the importance of monitoring the prevalence of COVID-19 in a population to be tested and proper validation of the test, to limit the potential for false-negative results. Validation studies to determine the optimal pool size by testing laboratories are recommended as the optimum approach. A safe, simple ‘two-stage pooling’ option has been indicated in this guidance to be used by laboratories until such validation can be achieved.

1. Introduction

In the absence of a vaccine and effective treatment, widespread testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), isolation of cases and tracing of contacts are key public health interventions to contain or slow down the progression of the pandemic, especially during the early phases. In collaboration with its partners, the Africa Centre for Disease Control and Prevention (Africa CDC) has supported the establishment and strengthening of reverse transcriptase–polymerase chain reaction (RT-PCR) testing capacity in Member States. This involves staff training, provision of test kits and supplies, and monitoring of testing quality. To date, 53 Member States have RT-PCR testing capacity for SARS-CoV-2, and the remaining two Member States are equipped with automated Xpert Xpress SARS-CoV-2 testing capability.
To support the scale-up of SARS-CoV-2 testing in the continent, Africa CDC launched the Partnership to Accelerate COVID-19 Testing (PACT) in April 2020 with an overall goal of conducting 10 million tests in 4–6 months and deployment of 1 million community health workers for contact tracing\textsuperscript{1,2}. To increase testing capacity in Member States, key strategies such as an expansion of testing to sub-national, research, veterinary, academic and private laboratories, expanding related human resource capacity, strengthening specimen collection, automation of testing technologies, and use of pooled sample testing were recommended\textsuperscript{3}.

Some countries have made remarkable progress in implementing the recommendations and were able to increase daily SARS-CoV-2 testing capacity significantly, in some cases from a few hundred to thousands. However, the current SARS-CoV-2 pandemic has stretched resources and laboratory infrastructure in many countries. As of 13 August 2020, a total of 9.8 million tests have been conducted in the continent with positivity rate of 11%. However, there is significant regional variation in the scale up of testing, with the Central Africa region contributing only 3.4% of the total tests, for example. Moreover, ten countries comprise 80% of the total testing capacity, indicating that many countries are still struggling to increase testing capacity as part of the pandemic response. Rapid scaling up of RT-PCR for SARS-CoV-2 testing in many countries has not been possible due to various constraints: lack of laboratory infrastructure; limited human resource; shortages of test kits and related supplies; challenges related to procurement; and weak surveillance systems. Maintaining a continuous supply of test kits and reagents is key to increasing SARS-CoV-2 testing capacity. In addition, the use of testing strategies such as pooled sample testing will facilitate economic use of scarce test kits and supplies and support expanded testing.
The pooling strategy could significantly increase the current testing capacity of laboratories in Africa with the currently available resources. This approach has great potential to accelerate the rate of testing and detection of SARS-CoV-2-infected patients in the community. It also considerably reduces the required resources for a given number of tests. Pooling allows testing of multiple samples obtained from multiple individuals using a single RT-PCR test. If the pool tests negative, all samples in a pool are declared negative. If the pooled sample tests positive, each sample can be individually re-tested to determine the positive or negative status of all samples in a given pool\textsuperscript{4,6}. Sub-pooling – or ‘three-stage hierarchical testing’ – can further optimize the use of resources in low SARS-CoV-2 prevalence settings\textsuperscript{5-7}. In sub-pooling, a large pool can be made from multiple sub-pools, further reducing the overall need for re-testing. If the large pool is positive, the sub-pools will be retested. If a given sub-pool tests positive, each of its members is tested separately. ‘Array testing’ is another approach that increases testing capacity further\textsuperscript{5}. Samples are arranged into a matrix-like structure, in which samples are pooled by rows and by columns to form pools. Samples at intersections of positive rows and columns are re-tested to determine an individual sample test outcome.

Pooling can be performed either before or after viral RNA extraction\textsuperscript{8}. Pooling clinical samples is preferred over the pooling of RNA extracts for additional resource saving. However, pooling RNA extracts may have additional benefits in terms of maintaining consistent sensitivity of the assay. However, RNA extraction kit shortage is one of the main causes of limited testing capacity in African settings. As a result, some laboratories use time-consuming manual extraction techniques. Performing viral RNA extraction once on multiple pooled samples can decrease use of extraction kits and other resources, and may be a more suitable approach for low- and middle-income countries.
Efficiency of the pooled sample testing strategy relies is determined by three key factors: pool size, sensitivity of the RT-PCR assay and prevalence of SARS-CoV-2 in the population\(^4\). The number of pooled samples should not significantly affect the initial sensitivity of the RT-PCR assay. Molecular assays with low sensitivity can generate false-negative results for the entire pool.

### 2. The test population for the pooled sample strategy

Sample pooling can be considered in the early phase of a local epidemic as the disease prevalence among symptomatic individuals is usually low (i.e. <1\%)\(^9\). In later pandemic stages, pooling is highly recommended for testing people who do not meet current individual testing criteria, including people without travel or any putative exposure history. It can be used effectively in low-risk groups or low-prevalence populations (≤1\%), particularly in resource-limited settings, as it may cut the testing requirements by as much as 70–80\%\(^{10,11}\).

In settings with limited resources, it is a practical strategy for identifying cases and transmission concealed in the community through large-scale screening, particularly in detecting asymptomatic and mild COVID-19 cases, including among high-risk groups\(^9,12\). The SARS-CoV-2 prevalence among a given asymptomatic population is estimated to be low (i.e. <1\%)\(^{13}\). Without a pooled sample approach in such settings, asymptomatic and mild cases may remain undetected in the community and the potential for transmission may not be avoided.

### 3. Maximizing number of samples for pooling

To maintain sensitivity of pooled sample testing, the number of tests required rises with increasing infection rates. This means that as SARS-CoV-2 prevalence decreases, the potential for savings in testing requirements increases\(^5,14\).
Accordingly, potential reductions in testing requirements are greater in homogeneous groups (i.e. pools of similar or connected people, such as families, neighbours, hospital employees, or workers in essential industries) than in heterogeneous pools. If one member of a homogeneous group is infected with SARS-CoV-2, the likelihood of other group members also being infected is relatively high. One modelling study showed that in a homogeneous group and a SARS-CoV-2 prevalence of 5–10%, for example, the statistically optimum pool size will be 10. If the prevalence is 1%, the ideal pool size increases to 25. In contrast, in an heterogeneous group with a prevalence of 5–10%, the appropriate pool size will be 5 or 3. Where the prevalence is 1%, the pool size will be 10$^{14}$. In general, generating homogeneous pools significantly lowers the number of tests required and saves substantial resources, particularly during ‘surge’ testing. In both situations, if we use a large pool size in high SARS-CoV-2 prevalence settings, the number of tests required slightly exceeds the number of tests required for individual testing.

The performance of the RT-PCR assay also affects the efficiency of the pooled testing strategy. Another modelling study showed pooled testing is an improvement over individual testing for a SARS-CoV-2 prevalence of less than 30% and can be considered as a viable alternative in settings with limited test availability. At various prevalence levels (e.g. 1%, 5% and 10%), the estimated number of false-negative results slightly increases when the sensitivity of the RT-PCR test becomes lower (from 100% to 60%). In settings where the SARS-CoV-2 prevalence is 5–10%, for example, if an assay with a sensitivity of ≥90% is used, the optimal pool size will be 5 or 4 with minimal risk of false negatives. Similarly, where the SARS-CoV-2 prevalence is 1%, the optimal pool size will be 11. The same modelling study showed that using a test with 70% sensitivity and a SARS-CoV-2 prevalence of 1%, the optimal pool size was 13, with an expected proportion of 0.07% false negative results. In general, with an optimal pool size of 13, high sensitivity RT-PCR tests
with a lower limit of detection ≥1100 RNA copies/ml will detect SARS-CoV-2 in pooled samples⁴.

Overall, the efficiency of the pooled testing strategy can be defined as the total number of pooled samples divided by the expected number of tests. In addition to the theoretical efficiency of sample pooling discussed above⁴,⁸, numerous studies have been conducted in different parts of the globe to validate the practical efficiency of pooling, although there is very limited evidence from Africa. Studies conducted in different settings with variable SARS-CoV-2 prevalence (<1% and 5%) and with pools ranging from 5 to 10 samples showed an improvement in RT-PCR testing efficiency with no or only slight loss of sensitivity of detecting SARS-CoV-2 (⁹–¹²,¹⁵). The Xpert Xpress SARS-CoV-2 test has also detected SARS-CoV-2 efficiently in the pool size of six samples with lower limit of detection ≥461 RNA copies/ml¹⁶. Furthermore, molecular tests targeting multiple SARS-CoV-2 genes may increase the efficiency of the detection of positive samples in pools¹⁵.

4. Recommendations

The pooled sample testing strategy should maintain the initial sensitivity of RNA extraction and RT-PCR tests, compared to individual testing. The pool size selected by laboratories shall not significantly affect the PCR cycle threshold (Ct) of an individual positive sample in the pooled samples. The dilution effect of pooling may increase the Ct value of a positive sample to an undetectable level and cause a false-negative result in samples with low viral load (i.e. Ct >35)¹⁰. In addition, an individual sample may lose its diagnostic integrity when pooled with other samples. There are other factors such as technical errors that could affect the reproducibility of the RT-PCR test. Test result interpretation may vary depending on the type of test platforms and kits used for RT-PCR testing¹¹,¹⁶. The interpretation of test results for pooled RT-PCR tests is similar to that with testing of individual samples. In individual tests,
only indeterminate results will require re-testing. Whereas in pooled sample testing, if the pool test result is positive or indeterminate, all samples in the pool must be re-tested individually.

In Africa, various types of molecular tests have been used for the detection of SARS-CoV-2. These molecular diagnostics use variable extraction kits, test kits, and/or platforms with the diagnostic accuracy varying accordingly. Some of the diagnostic technologies perform RNA extraction as part of testing in a closed system and do not require an extraction kit (e.g. GeneXpert)\(^\text{17}\). Hence, it is advisable to conduct a validation study by enrolling samples prospectively or using archived known positive and negative clinical samples retrospectively. It is important to consider that archived samples may affect the efficiency of the validation due to viral RNA degradation during storage and/or thawing. To validate the pooled testing strategy, the first step is selecting a range of pool sizes depending on the SARS-CoV-2 prevalence in the tested population, the accuracy of the assay, and the pooling algorithm. The positivity rate within the tested community can be taken as the prevalence of the disease. A useful web-based tool\(^\text{1}\) is available to determine the optimal pool size that provides the ideal reduction of the required number of tests\(^\text{11}\). It is also essential that the pool size selection considers the homogeneity and heterogeneity of the pools. It is also important to evaluate how the sensitivity of the assay can be affected by a particular pool size using defined assumptions. Finally, the optimal pool size that will not significantly decrease the sensitivity of the test – or shift the Ct value of an individual positive sample while pooling – should be selected. The evidence obtained from the validation study benefits the optimization and development of pooled testing protocol for the local context. This protocol should be applied for the same purpose of individual testing.

\(^{1}\text{See: https://www.chrisbilder.com/shiny/}.\)
Diagnostic laboratories may carefully consider the following recommendations for a simple two-stage pooling strategy if the validation of the pooling has not yet been conducted:

- In settings where the SARS-CoV-2 prevalence is <1%, and a molecular diagnostic test is used with a sensitivity of ≥96%, the optimal pool size can be 10.

- In settings where the SARS-CoV-2 prevalence is between ≥1% and ≤5%, and a molecular diagnostic test is used with a sensitivity of ≥96%, the optimal pool size can be 5.
References


