

Pooled Testing Can Increase SARS-CoV-2 Diagnostic Capacity in Kenya While Conserving Laboratory Resources

Key messages and recommendations

- We deployed a pooled testing strategy for SARS-CoV-2 at the KEMRI-CGMRC/KEMRI-Wellcome Trust Research Programme laboratories in Kilifi, when we were faced with a shortage of testing kits in mid-May 2020.
- Our testing capacity was improved by ~100% as a result of adoption of a six-sample pooled testing strategy and consumables used were reduced by ~50%.
- Pooled testing results in a slight decline in test sensitivity but reduces false positives because a second test is needed to confirm positives.
- Pooled testing is only advantageous while the prevalence of positives in test samples is low (<15%) and hence its deployment needs regular reassessment.

Background

International recommendations for the control of the COVID-19 pandemic emphasize the central role of laboratory testing for SARS-CoV-2, its etiological agent. Between 17th May and 26th June 2020, an average 2,833 SARS-CoV-2 laboratory tests were performed daily in Kenya (Figure 1). Scaling up of testing to enhance case detection, isolation, treatment and contact tracing has been a cornerstone strategy to manage the COVID-19 pandemic worldwide. Though the necessary equipment may be available in the country for scaling up testing, the actual testing capacity may be limited by the unavailability of reagents and qualified staff. Pooled testing, previously applied in blood banks to screen blood products for HIV-1, hepatitis B and C viruses, is a diagnostic approach where samples from multiple patients are combined and tested in a single reaction. If the reaction is positive, then samples that contributed to that reaction need to be retested individually. Many countries including the USA, Ghana, India, Ethiopia, Israel are using pooled testing to keep up with the high demand for SARS-CoV-2 testing. We evaluated whether pooled testing is a viable method to increase local testing capacity for SARS-CoV-2.

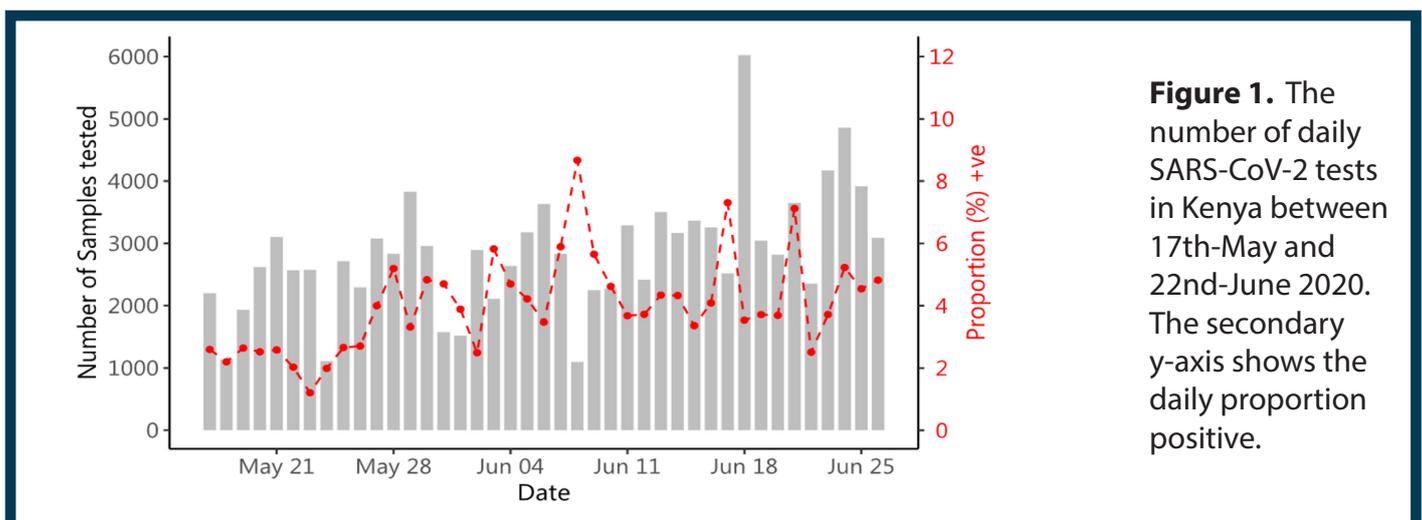


Figure 1. The number of daily SARS-CoV-2 tests in Kenya between 17th-May and 22nd-June 2020. The secondary y-axis shows the daily proportion positive.

KEMRI-Kilifi pooled testing experience

In mid-May 2020, we deployed the pooled testing strategy when we were faced with a shortage of high-throughput RNA extraction kits. Furthermore, during this period we were receiving >500 samples to test daily but we only had access to the low-throughput manual RNA extraction kits. To start pooled testing, we considered three key points (16): (a) the current real-time reverse transcription polymerase chain reaction (RT-PCR) diagnostic test limit of detection (b) the diagnostic test sensitivity and specificity and (c) the prevalence of the infection in our setting. The SARS-CoV-2 prevalence among tested samples in Kenya was ~4.0% (95% CI: 3.9-4.1%) for the period 17th May-26th June. Given this positivity rate, we adopted n=6 as our sample pool size (Figure 2).

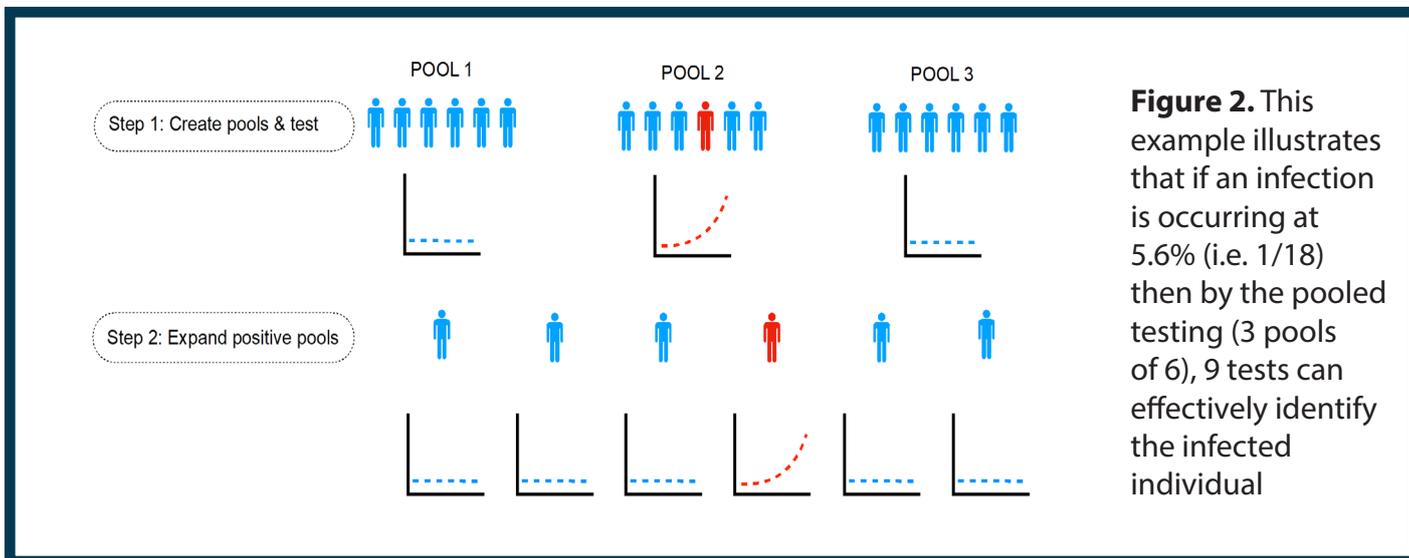


Figure 2. This example illustrates that if an infection is occurring at 5.6% (i.e. 1/18) then by the pooled testing (3 pools of 6), 9 tests can effectively identify the infected individual

Study Results

First, we assessed the impact of pooled testing on test sensitivity by combining a previously identified positive sample (that had been singly analyzed) with five negative samples. We replicated this 6 times. The positive samples were across a range of real-time RT-PCR Cycle thresholds (Ct) values (20.65-36.24). Lower Ct values indicate more strongly positive samples with more virus. The pools that included a strongly positive sample with a Ct value <33.0 also gave a positive result in the pools, while the pools including previously weakly positive samples that had a Ct value above 33.0 gave a negative result in the pools (Table 1). On retesting the individual samples for confirmation, all except the samples with the highest Ct value tested positive. This observation is consistent with previous literature on the lack of reproducibility of weak RT-PCR positives.

Table 1. Impact of pool testing on test results

Sample pool #	Original Ct	Pool Ct	Individual Ct
1 (Pool 1)	20.65	21.88	19.63
2 (Pool 2)	24.78	25.60	23.18
3 (Pool 3)	27.17	30.28	27.17
4 (Pool 4)	29.63	34.18	30.86
5 (Pool 5)	33.36	Negative	35.18
6 (Pool 6)	36.24	Negative	Negative

Table shows the Ct values obtained when the samples were tested the first time individually (Original Ct), when pool tested (Pool Ct) and when retested individually again (Individual Ct).

Second, we examined test results from 1500 samples tested in our laboratory between 11-12th, June 2020. The testing started with creating 250 pools (i.e. of 6 samples in each pool), 75 (30.0%) of which gave a positive result. The 75 positive pools were then expanded to 450 individual tests. From these, one or more positive samples were identified in 65 pools, a total of 112 positives (i.e. 7.5% of the original 1500 samples). The Ct value was 1.59 units higher for samples tested in pools compared with samples tested singly, indicating a slight decline of sensitivity in pools. Overall, to get results for 1500 samples we performed 700 tests, this saving 800 tests. We estimate the cost per test is ~\$6. Thus, by undertaking only 46.7% of the tests to identify the positives, using the pooled testing protocol we spent ~ \$4200 to test the 1500

samples compared with ~\$9000 if all samples are tested singly thus saving ~\$4800. Although two assays are required, because of the overall reduction in numbers of assays, the turnaround time could be faster and fewer staff are required to handle the laboratory tests.

Importantly, to calculate the optimal pool size we used the web-based shiny application from Christopher Bilder available at <https://www.chisbilder.com/shiny>. Although pooled testing can be applied for an infection whose prevalence is as high as 30%, it is most useful when the prevalence of the infection is low (typically <15%) (Table 2)(6).

Table 2. Comparison of Optimal Pool Size and prevalence rates on Test Efficiency

		number of Tests (%)	Efficiency (%)
1	11	80	400
3	6	67	200
5	5	57	133
7	4	50	100
10	4	41	69
15	3	28	39

Limitations

1. Due to sample dilution, there is a risk of missing weak positives but the repeat testing step reduces the chances of the laboratory releasing false positive results.
2. Although overall sample time was reduced, we are unable to “fast track” individual assays that are declared urgent by clinicians or public health officers where the initial test is positive.
3. Pooled testing is not suitable for screening populations at high risk of infection for example follow up samples from known infected individuals.

Conclusion

If pooled testing is adopted more widely across Kenya, there is potential that our testing capacity will significantly increase. The evaluation described here is based on the testing protocols at KEMRI CGMRC/ KEMRI-Wellcome Trust laboratories. For other labs to adopt the pooled testing procedure, assay-specific optimization is necessary. For instance, high-throughput systems like COBAS and Abbott combine processes that are conducted separately in KEMRI-Wellcome and therefore assay-specific evaluation will be required.

Recommendations

1. Pooling of positive samples with up to 5 negative samples works fine except for the very weak positives.
2. Laboratories across the country should be encouraged to consider SARS-CoV-2 pooled testing to save on testing kits and increase testing capacity.
3. For maximum returns from pool testing, the pool size should be kept under constant review if there are changes in the prevalence of the infection.

Related Publication

This brief is adapted from a research paper submitted under the title, “Pooled testing conserves SARS-CoV-2 laboratory resources and improves turn-around time: experience at KEMRI-Wellcome Trust Programme, Kenya.

For more information, contact **Dr Charles Nyaigoti** (Email: CNyaigoti@kemri-wellcome.org) or **Dr Isabella Ochola** (Email: LiOchola@kemri-wellcome.org) or **Dr Benjamin Tsofa** (Email: BTsofa@kemri-wellcome.org).