


















RESEARCH ARTICLE

Pooled testing conserves SARS-CoV-2 laboratory resources and improves test turn-around time: experience on the Kenyan Coast [version 1; peer review: 3 approved]

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Abstract

Background. International recommendations for the control of the coronavirus disease 2019 (COVID-19) pandemic emphasize the central role of laboratory testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent, at scale. The availability of testing reagents, laboratory equipment and qualified staff are important bottlenecks to achieving this. Elsewhere, pooled testing (i.e. combining multiple samples in the same reaction) has been suggested to increase testing capacities in the pandemic period. **Methods.** We discuss our experience with SARS-CoV-2 pooled testing using real-time reverse transcription polymerase chain reaction (RT-PCR) on the Kenyan Coast.

Results. In mid-May, 2020, our RT-PCR testing capacity for SARS-CoV-2 was improved by ~100% as a result of adoption of a six-sample pooled testing strategy. This was accompanied with a concomitant saving of ~50% of SARS-CoV-2 laboratory test kits at both the RNA extraction

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and RT-PCR stages. However, pooled testing came with a slight decline of test sensitivity. The RT-PCR cycle threshold value (ΔC_t) was ~1.59 higher for samples tested in pools compared to samples tested singly.

Conclusions. Pooled testing is a useful strategy to increase SARS-CoV-2 laboratory testing capacity especially in low-income settings.

Keywords

COVID-19, Pooled testing, SARS-CoV-2, Kilifi, Kenya





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Introduction

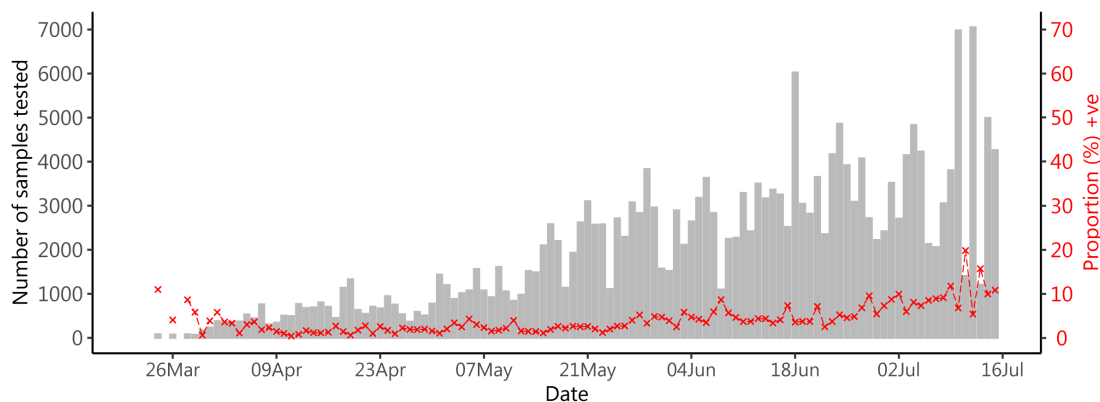
In Kenya, the first case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, the etiological agent of coronavirus disease 2019 (COVID-19), was confirmed on the 12th March 2020¹. Since then the number of confirmed cases has risen steadily, each day, and as of 15th July 2020, a total of 11,252 SARS-CoV-2 positives had been confirmed in the country from 225,495 samples tested, ~5.0% positivity rate overall². Scaling up of testing to enhance early case detection, isolation, treatment and to guide contact tracing has been a cornerstone strategy, worldwide, in managing the COVID-19 pandemic³. Between 15th May and 15th July 2020, an average 3,046 laboratory tests were performed daily in Kenya (Figure 1, panel A). Increasing the number of daily tests is a challenge for local laboratory capacity.

Real-time reverse transcription polymerase chain reaction (RT-PCR) is the gold standard method for SARS-CoV-2

diagnosis⁴. The diagnostic process is initiated by viral nucleic acid purification from a suspected patient sample, followed by concurrent target nucleic acid amplification and detection. Soon after 30th January 2020, when COVID-19 was declared a public health emergency of international concern, SARS-CoV-2 diagnostics were recognized as an important bottleneck in the efforts to effectively contain the epidemic⁵. Laboratory testing capacity may be limited by the unavailability of equipment, reagents and qualified staff. As a result, more efficient testing protocols have since been pursued to facilitate the mantra “test, trace, isolate and treat”. One such protocol is pooled testing^{5,6}.

Pooled testing is a diagnostic approach where samples from multiple patients are combined and analyzed in a single test reaction⁷. If the reaction is positive, then individual samples that contributed to that reaction need to be retested singly (Figure 2). Pooled testing was first used during world war II

A. Daily nationwide tests



B. Daily KEMRI-Kilifi tests

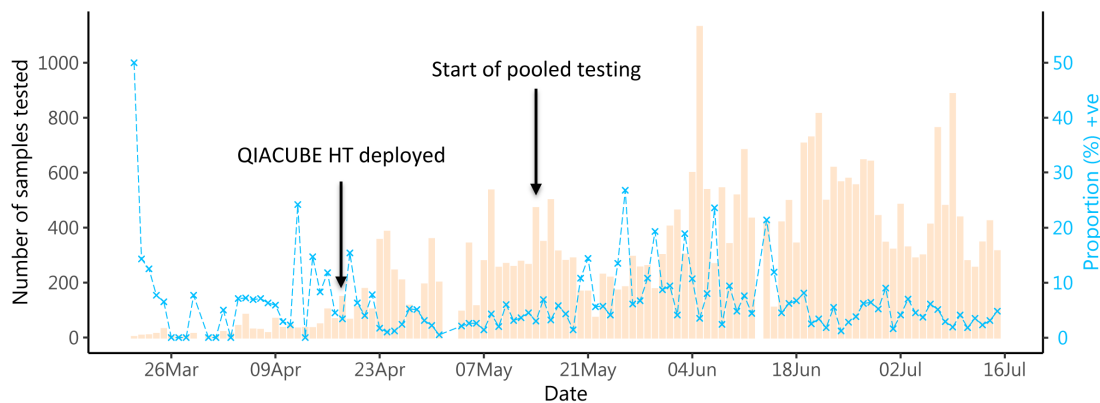


Figure 1. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) laboratory testing in Kenya between 21st March and 15th July 2020. Panel A, the bars show reported daily nationwide tests. Panel B, bars shows the daily tests undertaken at the KEMRI-Kilifi laboratory and when major protocol changes were implemented. In both panels the secondary y-axis shows the daily proportion of tests positive indicated by the dashed line.

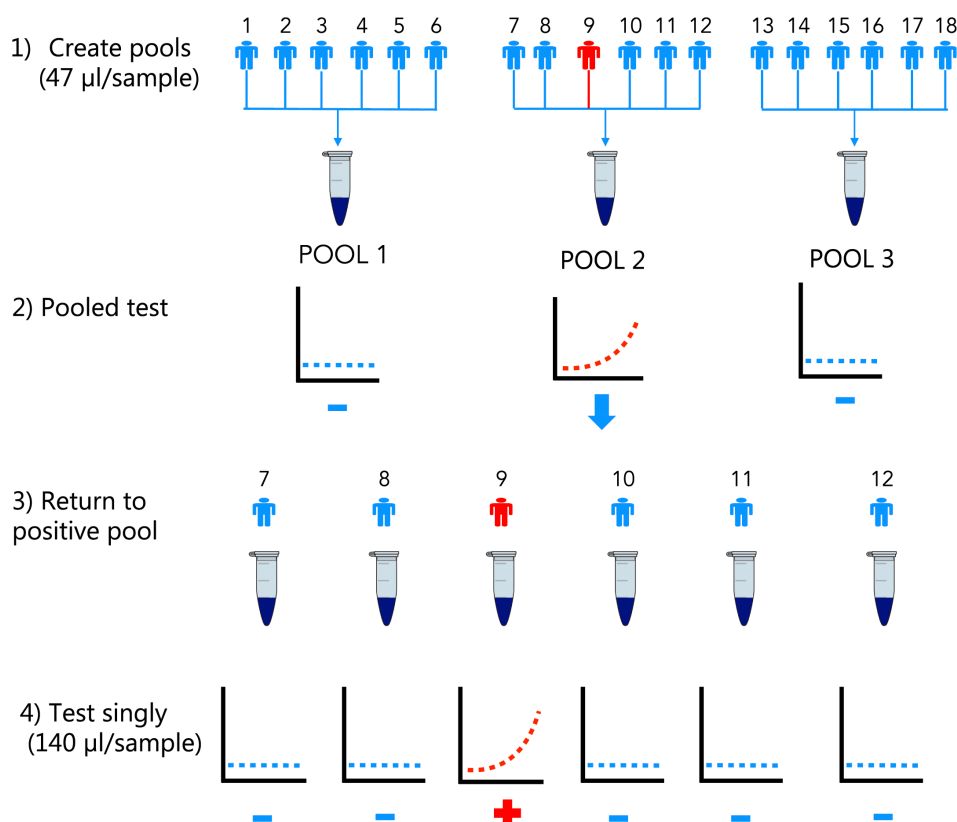


Figure 2. A schema of how the pooled testing strategy works. The example illustrates that if an infection is occurring at about 5.6% (i.e. 1/18) then by the pooled testing, a total of 9 tests can identify the infected individual. In Kenya, the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) positivity rate is ~5.0% thus pooled testing can conserve 50% of testing kits.

to efficiently identify syphilis infected military recruits⁸. More recently, this strategy has been applied in blood banks to screen blood products for HIV-1, hepatitis B and C viruses^{9,10}. Now, again, this strategy is finding application in identifying SARS-CoV-2 infected individuals in the ongoing COVID-19 pandemic^{7,11–15}.

Here, we evaluated whether pooled testing is a viable protocol for SARS-CoV-2 diagnosis in our Kenya setting and potentially other low-to-middle income settings across the globe. We first applied the pooled testing strategy in mid-May 2020 when we were receiving >300 SARS-CoV-2 test requests daily but had access only to the low-throughput manual RNA extraction kits (QIAamp Viral RNA Mini Kits). On applying pooled testing, we were able to keep up with the increasing volume of SARS-CoV-2 test requests daily and have henceforth maintained this strategy even with the high-throughput RNA extraction platforms due to the associated resource conservation.

Methods

Study site/location

This study was undertaken at the Kenya Medical Research Institute (KEMRI)-Wellcome Trust Research Programme (KWTRP) located in Kilifi town, on the Kenyan Coast between 21st March 2020 and 15th July 2020. Since the start of the

SARS-CoV-2 epidemic in Kenya, in March 2020, KEMRI-Kilifi has been supporting the County Health Department Rapid Response Teams (RRTs) in coastal region of Kenya in SARS-CoV-2 laboratory testing. Respiratory samples collected by the RRTs for testing are received in 2–3 ml of Universal Transport Media or Virus Transport Media. Data on the aggregated Kenya-wide daily SARS-CoV-2 laboratory tests and number of daily positives were compiled from the Kenya Ministry of Health (MoH) website, specifically the periodic COVID-19 situational reports and the [daily press releases](#).

Ethics and consent

The work was reviewed internally at KEMRI and considered part of the efforts to promptly develop efficient laboratory protocols for scaling up public health response to the COVID-19 pandemic. As a result, individual patient consent was considered unnecessary for these optimisation experiments. The national daily tally of SARS-CoV-2 tests done and number positive is freely available to the public at the MoH website inclusive of those from KEMRI laboratories.

Pooled testing protocol

For an optimal pooled testing protocol, there are three key considerations¹⁶: (i) the diagnostic protocol limit of detection (LoD) to ensure adequate sample volume is included in the

pools, (ii) the diagnostic test sensitivity and specificity and (iii) the prevalence of the infection to guide the optimal pool size (e.g. if infection prevalence reaches 30%, then pooling in groups of 3 would lead to most pools being positive and the need for individual testing, hence no gain in efficiency). In general, pooled testing is most useful when the prevalence of the infection is low (typically <15%)¹⁷.

To select the optimal pool size we used the web-based shiny application from Christopher Bilder available at <https://www.chrisbilder.com/shiny/> under Hierarchical testing. Assuming a SARS-CoV-2 prevalence of 4% in our query samples (see later in results section on observed test positivity rate, Figure 1), test sensitivity of 90%, test specificity of 98%¹⁸, adoption of a two stage pooling algorithm (Figure 2) and a pre-specified preferred pool size range of 3–10, the algorithm calculated the optimal testing configuration was a pool size of n=6 followed by individual testing of samples in positive pools.

Laboratory procedures

Our SARS-CoV-2 laboratory testing protocol has been described elsewhere¹⁹. Briefly, viral RNA purification from the raw samples was extracted using either of three commercial kits from QIAGEN (Manchester, UK); QIAamp Viral RNA Mini Kit (Catalogue # 52906), RNeasy® QIAcube® HT Kit (Catalogue # 74171) and QIASYMPHONY® RNA Kit (Catalogue # 931636). The manufacturer's instructions were followed for all the three kits. For the individual samples, viral RNA were extracted from starting volume of 140 µl of raw sample while for the pooled samples viral RNA were extracted from a starting volume of ~280 µl (each sample contributing 47 µl) (Figure 2). In both cases the purified RNA were collected in 60 µl of elution buffer.

RT-PCR was undertaken using primer/probes from the following four protocols, the details of which we described elsewhere¹⁹; (i) the Berlin (Charité)²⁰ (targeting E i.e. envelope gene, N i.e. nucleocapsid gene or RdRp i.e. RNA-dependent RNA-polymerase gene), (ii) European Virus Archive – GLOBAL (EVA-g) (targeting E or RdRp genes), (iii) Da An Gene Co. detection Kit (targeting N or ORF1ab) and Beijing Genomic Institute (BGI) RT-PCR kit (targeting ORF1ab). For the first two protocols only primer/probe mixes from the original protocol were used, as for the other RT-PCR components we used alternative RT-PCR reagents while the latter two are commercial kits that come with all RT-PCR components pre-mixed ready for RT-PCR running after addition of viral RNA extract from patient sample. Only EVA-g E gene protocol that was used in the experiments we described in the results section is further elaborated here in detail.

With the EVA-g assay, 4 µL of the purified RNA (pooled or individual samples) were mixed with 2.5 µl TaqMan™ Fast Virus 1-Step Master Mix (Applied Biosystems (ABI) Catalogue # 4444436), 1.75 µl E gene primer/ probe mix and 3.75 µl nuclease free water in a real-time PCR plate well. Three controls i.e. run positive control (PC), negative control (NC) and no template control (NTC), were included in every PCR plate for quality assurance and to aid in results interpretation. After sealing and

a short spin, the plate was loaded to an ABI 7500 instrument (ThermoFisher, USA). The thermocycling conditions used were; 50°C for 5 minutes, then 95°C for 20 seconds followed by 40 cycles of 95°C for 3 second and 58°C for 45 seconds. The amplification curves for all presumptive positive samples were visually inspected prior recording them as confirmed positives. A cycle threshold (Ct) of <38.0 was considered positive for pools and Ct of <37.0 for the individual samples. Lower Ct values indicate more strongly positive samples with more virus quantities.

Assessment of impact of pooled testing on assay sensitivity

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 Ct values (20.65–36.24) (Table 1²¹). The individual positive samples were retested again individually again to compare their repeat test Ct values with their previous test Ct values.

Data analysis

All numerical data manipulation was undertaken in STATA version 15.1. Positivity rate was calculated by dividing total positives by total samples tested over a specified period with the 95% confidence interval (CI) assuming a binomial distribution. Dispersion of Ct values were summarized using the median and interquartile range (IQR) values. Graphical presentations were generated in R version 3.5.0 using ggplot2 package version 2_3.2.1

Results

Optimal pool size and test turnaround

We started SARS-CoV-2 pooled testing on the Kenyan coast on the 14th May 2020 (Figure 1, panel B). The SARS-CoV-2 positivity rate among tested samples in the previous one month period (14th April–13th May 2020) across Kenya and in our laboratory was ~2.0% (95% CI: 1.8–2.1%) and ~3.3% (95% CI: 2.4–3.8%), respectively. Given the local and national positivity

Table 1. Impact of pooled testing on test sensitivity and positive sample 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 pooled testing was applied (Pool Ct) and when retested individually again (Individual Ct).

rate among tested samples during this period, and the anticipated increase in the following weeks, we inferred that an $n=6$ pool size was the optimal at that time point. Note that with similar assumptions of RT-PCR sensitivity and specificity parameters stated in the methods section, for SARS-CoV-2 positivity rates of: 5-7%; 8-15%; and 16-20% a pool size of: $n=5$, $n=4$ and $n=3$ would be recommended, respectively⁶.

The pooled testing strategy allowed us to screen 471 samples on the first day (14th May 2020) of deployment up from 264 the previous day, a 78% increase. Importantly the pooled testing protocol was using QIAamp manual Extraction Mini Kit for viral RNA purification a switch from the high-throughput QIAcube[®] HT Kit that we had deployed since 18th April 2020 (Figure 1, panel B).

Test sensitivity dynamics in pooled testing

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 1²¹. On repeat testing the previously positive individually, all confirmed positive results except one sample with the previous highest Ct value. This observation is consistent with previous

literature on the lack of reproducibility of weak RT-PCR positives especially those close to the test LoD^{22,23}.

Example pooled testing result in KEMRI-Kilifi laboratory

To further evaluate the benefit of pooled testing, we examined test results from 1500 samples tested in our laboratory between in the first week of June 2020. The testing started with creation of 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) (Figure 3, panel A).

On comparison of the Ct value differences (Δ Ct) in the pooled testing and individual sample testing (considering the strongest positive sample only where there were multiple positives in a pool), there was on average a 1.59 Ct value increase for the samples during pooled testing versus the same samples tested singly. Expanded pools tested negative for all 6 individual assays in 13.3% of instances (95% CI: 6.6-23.1%), despite initially testing positive. The Ct values for expanded pools testing negative for all 6 individual tests ranged 19.73 to 37.83 with median 30.45 (IQR: 26.1-35.66). For the pools where 1 or more positives

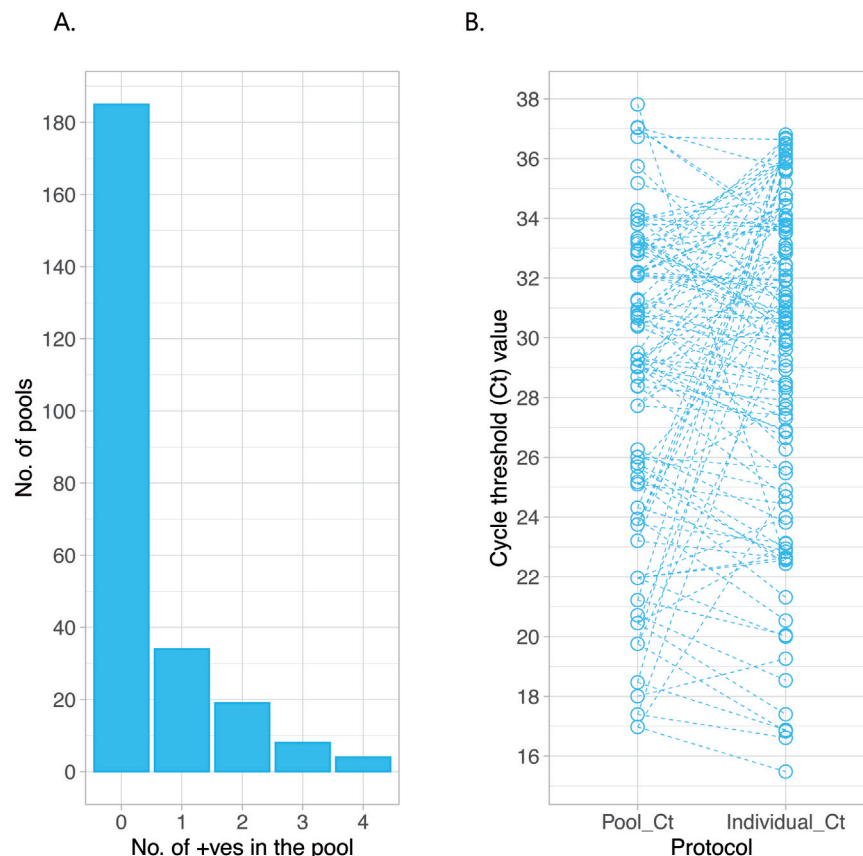


Figure 3. Summary results from the analysis of the 1500 samples analyzed at KEMRI-Kilifi using the pooled testing strategy. Panel A, shows the number of positives from the created pools. Panel B, comparison of Ct values from pooled tests versus individual sample tests.

were identified the Ct values ranged 16.97 to 37.81 with median 30.43 (IQR: 25.68-32.90). False-positive results during pooled testing may arise as technical artifacts of degraded probe, primer/probe cross-reaction with non-SARS-CoV-2 sequences in some samples or technical cross-contamination/mislabeling during sample processing²⁴.

Resources conserved in pooled testing

Overall, in the above example, to get results for 1500 samples we performed 700 tests (RNA extraction and RT-PCR). We estimated that in our laboratory, it costed ~ 6 United States Dollars (USD) per SARS-CoV-2 test. Thus, by undertaking only 46.7% of the tests to identify the positives, using the pooled testing protocol we spent ~ 4200 USD to test the 1500 samples down from ~9000 USD if all samples are tested singly thus saving ~4800 USD. Although two assays were required, because of the overall reduction in numbers of assays, the turnaround time was faster and fewer staff were required to handle the laboratory tests when using the pooled testing approach.

Conclusions

Pooled testing can yield significant savings of test kits resources while effectively identifying infected SARS-CoV-2 individuals in the population rapidly. This protocol is especially relevant in low-to-middle income settings as testing resources are mostly dependent on limited purchased imports or donations. The strategy further increases test specificity (positives are tested twice) limiting false positives²⁵. However, due to sample dilution, there is a risk of missing weak positives during the first step of pooled testing. Although overall the sample handling time was reduced, it was difficult to “fast track” individual assays that were declared urgent by clinicians or public health officers where the initial pooled test is positive. As the COVID-19 pandemic evolves, the pool size used by a testing laboratory

should be kept under constant review and adjusted if there are changes in the prevalence of the infection in the target population or test accuracy characteristics.

Data availability

Underlying data

Replication Data for: Pooled testing conserves SARS-CoV-2 laboratory resources and improves turn-around time: experience at KEMRI-Wellcome Trust Programme, Kenya. <https://doi.org/10.7910/DVN/I4XUC5>²¹

This project contains the following underlying data:

- CAgoti_SARSCoV2_Lab_Experience_Codebook.pdf (Codebook for datasets)
- CAgoti_SARSCoV2_Lab_Experience_Readme.txt (Data description and usage instructions)
- datafiles.zip (Analysis datasets)
- scripts.zip (Analysis scripts)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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James A Hay 

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Agoti *et al.* provide a timely and important report on the implementation of pooled SARS-CoV-2 PCR testing on the Kenyan Coast. The manuscript describes the epidemiological context and pooling strategy concisely and clearly. The authors find that pooling drastically increases testing throughput and saves on number of test kits used. Given the hesitancy of many settings to implement pooled testing for fears of logistical challenges and reduced sensitivity, this is an important success story that should be useful to many low-and-middle income settings (and indeed high-income settings!). I really enjoyed reading this paper!

1. The title and results suggest that pooling reduced the test turnaround time. However, I can see no results to support this claim (other than the final, qualitative results sentence). This is a key (potential) benefit of pooling, so please could you provide these results?
2. It would be useful to provide some context on why these tests were carried out. Presumably all of these samples were from a clinical setting, where the considerations for sensitivity (and specificity) may be different than e.g. surveillance or routine screening of HCWs.
3. "... by combining a previously identified positive sample ... we replicated this 6 times". This phrasing suggests the same positive sample was diluted 6 different times. Please rephrase to make clear that these were 6 distinct samples across a range of Ct values. Also, were these re-tested samples subjected to an additional freeze-thaw cycle which might degrade RNA?
4. Section "Example pooled testing result in KEMRI-Kilifi laboratory": "... in our laboratory between in the first week of June 2020". Typo here (remove between). Also, "The testing started with creation of...", missing "the".
5. It would be interesting to discuss where some of the Ct values actually increased from the pooled to individual testing. This could help give an idea of how much testing variation

there is in addition to reduced sensitivity through dilution or may simply be due to the presence of both low and high Ct samples in the pool.

6. For the 13.3% of positive pools which had no individually positive samples, was there any retesting or were they assumed false positives? It seems unlikely that all of these pools were false positives (indeed most were likely false negative individual tests). Also, bringing this up here seems a bit at odds with the discussion sentence "The strategy further increases test specificity...".
7. I think it may be useful to discuss some of the practical lessons learned here. For example, was it easy for staff to adapt their workflow to implement pooling rather than individual testing? How much longer did it take to test a batch using pooling vs. individual testing? I'm sure there are many laboratories around the world that would be interested to hear about the more qualitative lessons learned here.
8. The conclusions are a bit brief. For example, I think readers would like to hear more about the difficulty in fast tracking individual assays. As suggested above, context regarding where and why samples were tested is important, as speed and accuracy requirements may depend on why a test is carried out.
9. As suggested by another reviewer, it would be interesting to see if the number of validation tests required tracked changing % positive.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epidemiological modeling. I have focused on strategies to improve and harness PCR testing during the COVID-19 pandemic (including pooling).

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 26 October 2020

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Kenny Voon 

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The manuscripts is generally well written. Agoti *et al.* describe their experience with SARS-CoV-2 pooled testing in KIMRI-Kilifi. The authors point out that the rational of pooled testing is to conserve resources and had calculated pooling size according to prevalence rate.

However, several following points in the manuscript should be improved/ clarified.

1. The authors should clarify whether 6- samples-pooled testing were conducted until 16 Jul. 2020. Based on Figure 1, the percentage of positives seems to have sporadic increase from 21 May until 18 June, which may lead to increase of prevalence.
2. Figure 2B show some pooled CT values are lower than individual Ct values and vise versa. This does not support fully the statement of "RT-PCR cycle threshold value was -1.59 higher for samples tested in pools compared to samples tested singly". The authors should clarify why some pooled CT values are lower than individual Ct values. Were these the effect of several positives in one-pool?
3. Were the pooling being done randomly or by cluster or stratified?

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Virology, COVID-19 pooling,

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 26 August 2020

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Agoti *et al.* share their experience with SARS-CoV-2 pooled testing using real-time reverse transcription polymerase chain reaction (RT-PCR) on the Kenyan Coast. The authors evaluated the viability of pooled testing approach for SARS-CoV-2 diagnosis in low-to-middle income settings such as Kenya. To achieve this, the authors utilized a total of 1500 respiratory samples collected in the coastal region of Kenya during the first week of June 2020. A total of 250 pools (each comprising 6 samples) were subjected to RT-PCR, followed by individual testing of samples in positive pools. By employing this strategy, only 700 tests (including RNA extraction and RT-PCR) were conducted to get results for the 1500 samples, translating into a cost reduction of 4,800 USD. Although data analysis reveals increased test specificity, the strategy has an associated risk of reduced sensitivity which might result in false negatives.

The study has been well conducted and the manuscript well written. The findings of this study are timely in light of the global COVID-19 pandemic that has created an urgent demand for accurate rapid diagnostic strategies to allow for prompt clinical and well-tuned public health interventions. The pandemic has resulted in unprecedented demand on the RT-PCR testing capacity of all countries. Demand for testing has been coupled with a global shortage of commercial kits, reagents, consumables, disruptions in the global transport networks, and exacerbated by international competition for testing resources. Accordingly, even many high-income countries have inadequate RT-PCR testing capacity to effectively suppress ongoing transmission, and most low and middle-income countries (LMICs) are unlikely to be able to establish adequate RT-PCR capacity in the immediate future. Thus, the pooled testing strategy for SARS-CoV-2 offers an

attractive solution in molecular testing especially for LMICs.

However, **minor revisions** are required to accept the manuscript for indexing:

1. The authors described the specificity and sensitivity of the pooled testing strategy for SARS-CoV-2 in general terms. Please provide calculated figures for these measures of test reliability.
2. The authors note in their conclusion that, "due to sample dilution, there is a risk of missing weak positives during the first step of pooled testing." It will be useful for the authors to explain the implication of such reduced sensitivity and if there are any mitigation measures that can be employed to help improve the reliability of the test.
3. Was there any method to the pooling of samples? Was it random or non-random?
4. Were the samples from symptomatic or asymptomatic patients?
5. What is the effect of viral loads on pool size and test performance?
6. Please correct a few typographical errors especially under the section, "Example pooled testing result in KEMRI-Kilifi laboratory."

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious diseases, point-of-care diagnostics development, clinical medicine

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
