


















## 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]

Charles N. Agoti <sup>1,2</sup>, Martin Mutunga<sup>1</sup>, Arnold W. Lambisia <sup>1</sup>, Domtila Kimani<sup>1</sup>, Robinson Cheruiyot<sup>1</sup>, Patience Kiyuka <sup>1</sup>, Clement Lewa<sup>1</sup>, Elijah Gicheru<sup>1</sup>, Metrine Tendwa<sup>1</sup>, Khadija Said Mohammed <sup>1</sup>, Victor Osoti<sup>1</sup>, Johnstone Makale <sup>1</sup>, Brian Tawa<sup>1</sup>, Calleb Odundo<sup>1</sup>, Wesley Cheruiyot<sup>1</sup>, Wilfred Nyamu<sup>1</sup>, Wilson Gumbi<sup>1</sup>, Jedidah Mwacharo<sup>1</sup>, Lydia Nyamako<sup>1</sup>, Edward Otieno <sup>1</sup>, David Amadi <sup>1</sup>, Janet Thoya<sup>1</sup>, Angela Karani<sup>1</sup>, Daisy Mugo<sup>1</sup>, Jennifer Musyoki<sup>1</sup>, Horace Gumba <sup>1</sup>, Salim Mwarumba<sup>1</sup>, Bonface M. Gichuki <sup>1</sup>, Susan Njuguna<sup>1</sup>, Debra Riako<sup>1</sup>, Shadrack Mutua<sup>1</sup>, John N. Gitonga <sup>1</sup>, Yiakon Sein<sup>1</sup>, Brian Bartilol <sup>1</sup>, Shaban J. Mwangi <sup>1</sup>, Donwilliams O. Omuoyo<sup>1</sup>, John M. Morobe <sup>1</sup>, Zaydah R. de Laurent <sup>1</sup>, Philip Bejon <sup>1,3</sup>, Lynette Isabella Ochola-Oyier<sup>1</sup>, Benjamin Tsofa<sup>1</sup>

<sup>1</sup>Kenya Medical Research Institute-Wellcome Trust Research Programme, Centre for Geographic Medicine Research, Kilifi, Kenya

<sup>2</sup>Department of Biomedical Sciences, Pwani University, Kilifi, Kenya

<sup>3</sup>Nuffield Department of Medicine, Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, University of Oxford, Oxford, UK

**V1** First published: 06 Aug 2020, 5:186  
<https://doi.org/10.12688/wellcomeopenres.16113.1>  
 Latest published: 03 Feb 2021, 5:186  
<https://doi.org/10.12688/wellcomeopenres.16113.2>

## 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

## Open Peer Review

Reviewer Status 

Invited Reviewers

1 2 3

version 2

(revision)

03 Feb 2021

version 1

06 Aug 2020



report



report



report

1. Ernest Wandera Apondi, KEMRI/Nagasaki

University, Nairobi, Kenya

~50% of SARS-CoV-2 laboratory test kits at both the RNA extraction and RT-PCR stages. However, pooled testing came with a slight decline of test sensitivity. The RT-PCR cycle threshold value ( $\Delta C_t$ ) was ~1.59 higher for samples tested in pools compared to samples tested singly.

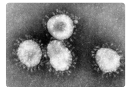
**Conclusions.** Pooled testing is a useful strategy to increase SARS-CoV-2 laboratory testing capacity especially in low-income settings.

### Keywords

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




This article is included in the [KEMRI | Wellcome Trust](#) gateway.




This article is included in the [Coronavirus \(COVID-19\)](#) collection.

Mount Kenya University, Thika, Kenya

**Jesse Gitaka** , Mount Kenya University, Thika, Kenya

Mount Kenya University, Thika, Kenya

2. **Kenny Voon** , School of Medicine, International Medical University, Kuala Lumpur, Malaysia

3. **James A Hay** , Centre for Communicable Disease Dynamics, Department of Epidemiology, Harvard School of Public Health, Boston, USA

Any reports and responses or comments on the article can be found at the end of the article.

**Corresponding author:** Charles N. Agoti ([cnyaigoti@kemri-wellcome.org](mailto:cnyaigoti@kemri-wellcome.org))

**Author roles:** **Agoti CN:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Mutunga M:** Investigation, Methodology, Project Administration, Writing – Review & Editing; **Lambisia AW:** Investigation, Writing – Review & Editing; **Kimani D:** Investigation, Writing – Review & Editing; **Cheruiyot R:** Investigation, Writing – Review & Editing; **Kiyuka P:** Investigation, Writing – Review & Editing; **Lewa C:** Investigation, Writing – Review & Editing; **Gicheru E:** Investigation, Writing – Review & Editing; **Tendwa M:** Investigation, Writing – Review & Editing; **Said Mohammed K:** Investigation, Writing – Review & Editing; **Osoti V:** Investigation, Writing – Review & Editing; **Makale J:** Investigation, Writing – Review & Editing; **Tawa B:** Investigation, Writing – Review & Editing; **Odundo C:** Investigation, Writing – Review & Editing; **Cheruiyot W:** Investigation, Writing – Review & Editing; **Nyamu W:** Investigation, Writing – Review & Editing; **Gumbi W:** Investigation, Writing – Review & Editing; **Mwacharo J:** Investigation, Writing – Review & Editing; **Nyamako L:** Investigation, Writing – Review & Editing; **Otieno E:** Data Curation, Writing – Review & Editing; **Amadi D:** Data Curation, Writing – Review & Editing; **Thoya J:** Data Curation, Writing – Review & Editing; **Karani A:** Investigation, Writing – Review & Editing; **Mugo D:** Investigation, Writing – Review & Editing; **Musyoki J:** Investigation, Project Administration, Writing – Review & Editing; **Gumba H:** Investigation, Writing – Review & Editing; **Mwarumba S:** Project Administration, Writing – Review & Editing; **M. Gichuki B:** Investigation, Writing – Review & Editing; **Njuguna S:** Investigation, Writing – Review & Editing; **Riako D:** Investigation, Writing – Review & Editing; **Mutua S:** Investigation, Writing – Review & Editing; **Gitonga JN:** Investigation, Writing – Review & Editing; **Sein Y:** Investigation, Writing – Review & Editing; **Bartilol B:** Investigation, Writing – Review & Editing; **Mwangi SJ:** Investigation, Writing – Review & Editing; **O. Omuoyo D:** Investigation, Writing – Review & Editing; **M. Morobe J:** Investigation, Writing – Review & Editing; **de Laurent ZR:** Investigation, Writing – Review & Editing; **Bejon P:** Conceptualization, Funding Acquisition, Project Administration, Resources, Supervision, Writing – Review & Editing; **Ochola-Oyier LI:** Conceptualization, Investigation, Methodology, Project Administration, Supervision, Validation, Writing – Review & Editing; **Tsofa B:** Conceptualization, Project Administration, Supervision, Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

**Grant information:** This work was supported by the Wellcome Trust through a Wellcome Intermediate Fellowship to LIOO which also supports VO [107568]; Core Support for the East African Major Overseas Programme [203077]; and support to the Initiative to Develop African Research Leaders (IDeAL) [107769]. This work was also supported by the African Academy of Sciences (AAS) through support to CA and PK as part of the IDeAL programme. IDeAL is a programme of the The Developing Excellence in Leadership, Training and Science (DELTA) Africa [DEL-15-003]. The DELTA Africa Initiative is an independent funding scheme of the AAS's Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency). Support was also provided by the United Kingdom Department for International Development (DFID) in conjunction with Wellcome [107769 and 203077] and the National Institute for Health Research (NIHR) [project reference 17/63/82] using UK aid from the UK Government to support global health research. The views expressed in this report are those of the authors and not necessarily those of AAS, NEPAD Agency, The Wellcome, NIHR or the UK government.

*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

**Copyright:** © 2020 Agoti CN *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**How to cite this article:** Agoti CN, Mutunga M, Lambisia AW *et al.* **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]** Wellcome Open Research 2020, 5:186 <https://doi.org/10.12688/wellcomeopenres.16113.1>

**First published:** 06 Aug 2020, 5:186 <https://doi.org/10.12688/wellcomeopenres.16113.1>

## Introduction

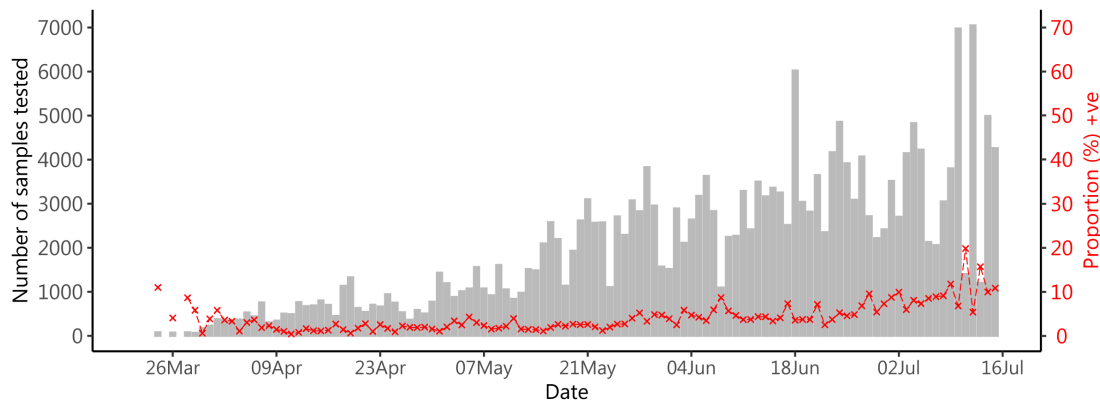
In Kenya, the first case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, the etiological agent of coronavirus disease 2019 (COVID-19), was confirmed on the 12<sup>th</sup> March 2020<sup>1</sup>. Since then the number of confirmed cases has risen steadily, each day, and as of 15<sup>th</sup> 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 overall<sup>2</sup>. 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 pandemic<sup>3</sup>. Between 15<sup>th</sup> May and 15<sup>th</sup> 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

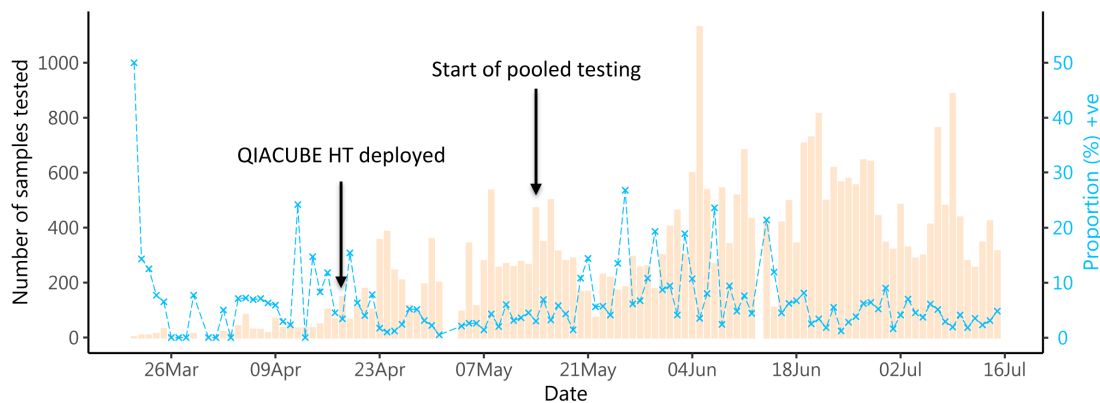
diagnosis<sup>4</sup>. The diagnostic process is initiated by viral nucleic acid purification from a suspected patient sample, followed by concurrent target nucleic acid amplification and detection. Soon after 30<sup>th</sup> January 2020, when COVID-19 was declared a public health emergency of international concern, SARS-CoV-2 diagnostics were recognized as an important bottleneck in the efforts to effectively contain the epidemic<sup>5</sup>. 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<sup>5,6</sup>.

Pooled testing is a diagnostic approach where samples from multiple patients are combined and analyzed in a single test reaction<sup>7</sup>. 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

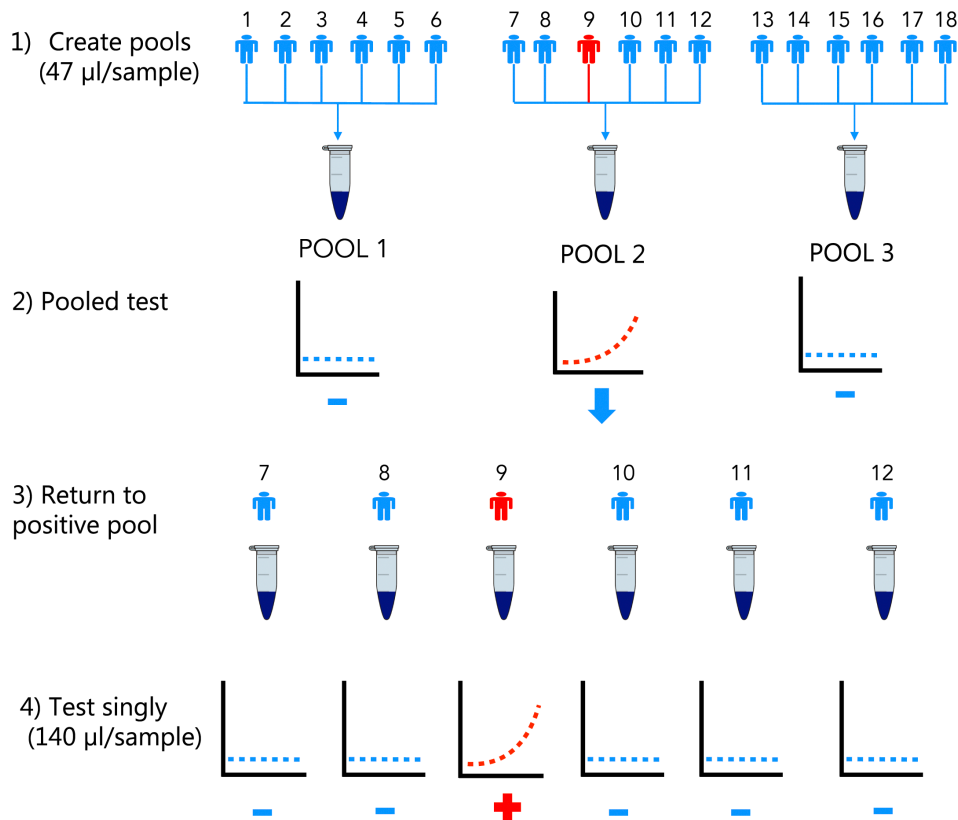
### A. Daily nationwide tests



### B. Daily KEMRI-Kilifi tests



**Figure 1. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) laboratory testing in Kenya between 21<sup>st</sup> March and 15<sup>th</sup> 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 recruits<sup>8</sup>. More recently, this strategy has been applied in blood banks to screen blood products for HIV-1, hepatitis B and C viruses<sup>9,10</sup>. Now, again, this strategy is finding application in identifying SARS-CoV-2 infected individuals in the ongoing COVID-19 pandemic<sup>7,11-15</sup>.

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 21<sup>st</sup> March 2020 and 15<sup>th</sup> 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 considerations<sup>16</sup>: (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%)<sup>17</sup>.

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%<sup>18</sup>, 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 elsewhere<sup>19</sup>. 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<sup>®</sup> QIAcube<sup>®</sup> HT Kit (Catalogue # 74171) and QIASYMPHONY<sup>®</sup> 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 elsewhere<sup>19</sup>; (i) the Berlin (Charité)<sup>20</sup> (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<sup>™</sup> 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 1<sup>21</sup>). 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 14<sup>th</sup> May 2020 (Figure 1, panel B). The SARS-CoV-2 positivity rate among tested samples in the previous one month period (14<sup>th</sup> April-13<sup>th</sup> 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<sup>‡</sup>.**

| 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      |

<sup>‡</sup>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, respectively<sup>6</sup>.

The pooled testing strategy allowed us to screen 471 samples on the first day (14<sup>th</sup> 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<sup>®</sup> HT Kit that we had deployed since 18<sup>th</sup> April 2020 (Figure 1, panel B).

### Test sensitivity dynamics in pooled testing

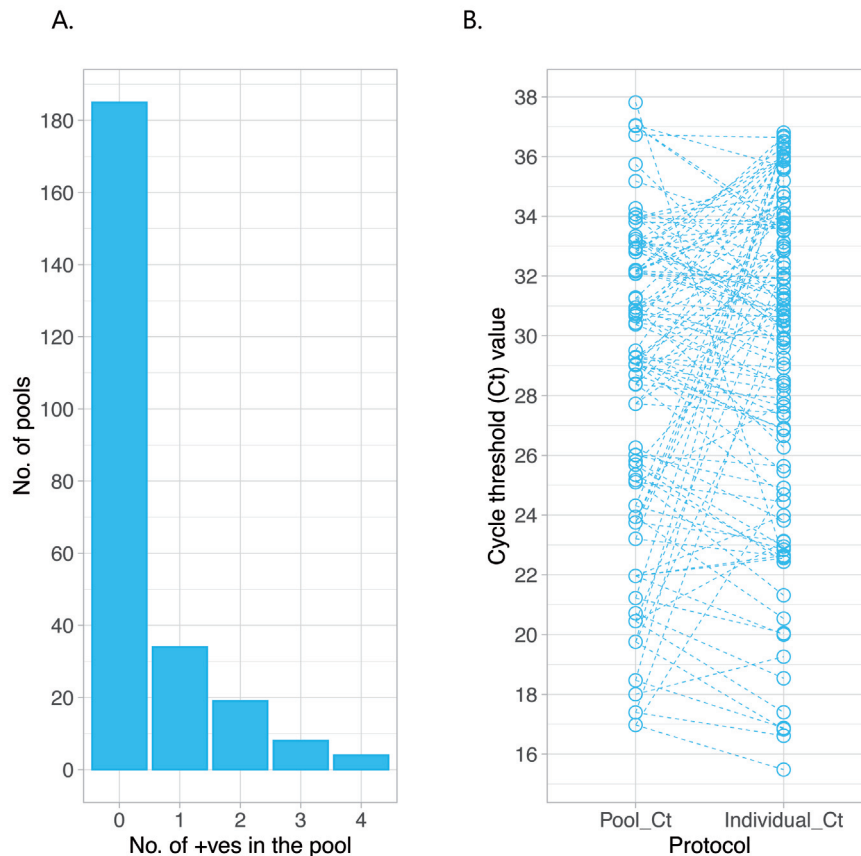
The pools that included a strongly positive sample with a Ct value  $<33.0$  also gave a positive result in the pools, while the pools including previously weakly positive samples that had a Ct value above 33.0 gave a negative result in the pools Table 1<sup>21</sup>. On repeat testing the previously 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<sup>22,23</sup>.

### 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 ( $\Delta$ 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/mislabeled during sample processing<sup>24</sup>.

### 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 positives<sup>25</sup>. 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><sup>21</sup>

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 \(CC-BY 4.0\)](#).

### Acknowledgments

We are thankful to the members of the Ministry of Health Rapid Response Teams in coastal Kenya counties who collected the patient samples. We grateful to the European Virus Archive Global for kindly providing us the SARS-CoV-2 primers and probes used in our COVID-19 testing. This manuscript was submitted for publication with the permission of the Director KEMRI.

### References

1. MoH: **First Case of Coronavirus Disease Confirmed in Kenya**. MoH, Editor. Ministry of Health: Press release. 2020; 1–3. [Reference Source](#)
2. MoH: **COVID-19 Outbreak in Kenya**. In *Daily Situation Report 122*, E.O. Center, Editor. MoH: MoH website. 2020; 1–4. [Reference Source](#)
3. Nanyingi M: **The evolution of the COVID-19 pandemic in Kenya**. T.R.S.o.T.M.a. Hygiene, Editor. 2020. [Reference Source](#)
4. Tang YW, Schmitz JE, Persing DH, *et al.*: **Laboratory Diagnosis of COVID-19: Current Issues and Challenges**. *J Clin Microbiol.* 2020; **58**(6): e00512-20. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
5. Esbin MN, Whitney ON, Chong S, *et al.*: **Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection**. *RNA.* 2020; **26**(7): 771–783. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
6. Abdalhamid B, Bilder CR, McCutchen EL, *et al.*: **Assessment of Specimen Pooling to Conserve SARS CoV-2 Testing Resources**. *Am J Clin Pathol.* 2020; **153**(6): 715–718. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
7. Eberhardt JN, Breuckmann NP, Eberhardt CS: **Multi-Stage Group Testing Improves Efficiency of Large-Scale COVID-19 Screening**. *J Clin Virol.* 2020; **128**: 104382. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
8. Dorfman R: **The Detection of Defective Members of Large Populations**. *Ann Math Stat.* 1943; **14**: 436–440. [Publisher Full Text](#)
9. Westreich DJ, Huddgens MG, Fiscus SA, *et al.*: **Optimizing Screening for Acute Human Immunodeficiency Virus Infection with Pooled Nucleic Acid Amplification Tests**. *J Clin Microbiol.* 2008; **46**(5): 1785–1792. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
10. Cardoso MS, Koerner K, Kubanek B: **Mini-pool screening by nucleic acid testing for hepatitis B virus, hepatitis C virus, and HIV: preliminary results**. *Transfusion.* 1998; **38**(10): 905–7. [PubMed Abstract](#) | [Publisher Full Text](#)
11. Ihekweazu C, Agogo E: **Africa's response to COVID-19**. *BMC Med.* 2020; **18**(1): 151. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
12. Mulu A, Alemayehu DH, Alemu F, *et al.*: **Evaluation of Sample Pooling for Screening of SARS CoV-2**. *medRxiv.* 2020. [Publisher Full Text](#)
13. Yelin I, Aharony N, Tamar ES, *et al.*: **Evaluation of COVID-19 RT-qPCR test in multi-sample pools**. *Clin Infect Dis.* 2020; ciaa531. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
14. Lohse S, Pfuhl T, Berkó-Göttel B, *et al.*: **Pooling of samples for testing for SARS-CoV-2 in asymptomatic people**. *Lancet Infect Dis.* 2020; S1473-3099(20)30362-5. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)



15. Hogan CA, Sahoo MK, Pinsky BA: **Sample Pooling as a Strategy to Detect Community Transmission of SARS-CoV-2.** *JAMA.* 2020; **323**(19): 1967–1969. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
16. Pouwels KB, Roope LSJ, Barnett A, *et al.*: **Group Testing for SARS-CoV-2: Forward to the Past?** *Pharmacoecon Open.* 2020; **4**(2): 207–210. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Abdalhamid B, Bilder CR, McCutchen EL, *et al.*: **Assessment of Specimen Pooling to Conserve SARS CoV-2 Testing Resources.** *Am J Clin Pathol.* 2020; **153**(6): 715–718. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
18. Böger B, Fachi MM, Vilhena RO, *et al.*: **Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19.** *Am J Infect Control.* 2020; S0196-6553(20)30693-3. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
19. Mohammed KS, de Laurent ZR, Omuoyo DO, *et al.*: **An optimisation of four SARS-CoV-2 qRT-PCR assays in a Kenyan laboratory to support the national COVID-19 rapid response teams [version 1; peer review: awaiting peer review].** *Wellcome Open Res.* 2020; **5**: 162. [Publisher Full Text](#)
20. Corman VM, Landt O, Kaiser M, *et al.*: **Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR.** *Euro Surveill.* 2020; **25**(3): 2000045. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
21. Agoti CN: **Replication Data, for: Pooled testing conserves SARS-CoV-2 laboratory resources and improves turn-around time: experience at KEMRI-Wellcome Trust Programme, Kenya.** *Harvard Dataverse.* 2020. <http://www.doi.org/10.7910/DVN/I4XUC5>
22. Wölfel R, Corman VM, Guggemos W, *et al.*: **Virological assessment of hospitalized patients with COVID-2019.** *Nature.* 2020; **581**(7809): 465–469. [PubMed Abstract](#) | [Publisher Full Text](#)
23. Arnaout R, Lee RA, Lee GR, *et al.*: **SARS-CoV2 Testing: The Limit of Detection Matters.** *bioRxiv.* 2020; 2020.06.02.131144. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
24. Katz AP, Civantos FJ, Sargi Z, *et al.*: **False-positive reverse transcriptase polymerase chain reaction screening for SARS-CoV-2 in the setting of urgent head and neck surgery and otolaryngologic emergencies during the pandemic: Clinical implications.** *Head Neck.* 2020; **42**(7): 1621–1628. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
25. Pikovski A, Bentele K: **Remarks on pooling Coronavirus tests.** *medRxiv.* 2020. [Publisher Full Text](#)

# Open Peer Review

Current Peer Review Status:   

---

## Version 1

Reviewer Report 30 October 2020

<https://doi.org/10.21956/wellcomeopenres.17686.r40819>

© 2020 Hay J. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

 **James A Hay** 

Centre for Communicable Disease Dynamics, Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA

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

<https://doi.org/10.21956/wellcomeopenres.17686.r40816>

© 2020 Voon K. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



**Kenny Voon** 

School of Medicine, International Medical University, Kuala Lumpur, Malaysia

The manuscript 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 rationale 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 vice 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.**

Author Response 28 Oct 2020

**Arnold Wasike Lambisia**, Kenya Medical Research Institute-Wellcome Trust Research Programme, Centre for Geographic Medicine Research, Kilifi, Kenya

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.**

*Our response: Yes, the n=6 pool size was maintained from 14<sup>th</sup> May to 31 July 2020 as the high positivity rate spikes during this period were short-lived rather than sustained.*

1. **Figure 2B show some pooled CT values are lower than individual Ct values and vice 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?**

*Our response: The reviewer is correct in their interpretation i.e. such was observed in pools with more than one positive. In the result section, we did point this out: "On comparison of the Ct value differences ( $\Delta Ct$ ) 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. (Note: The reviewer is referring to figure 3B rather than 2B which is relevant to the question)*

1. **Were the pooling being done randomly or by cluster or stratified?**

*Our response: Same question was raised by reviewer #1 above (question #3), so same answer. We have updated this information into the revised version of the manuscript*

**Competing Interests:** No competing interests were disclosed.

Reviewer Report 26 August 2020

<https://doi.org/10.21956/wellcomeopenres.17686.r39878>

© 2020 Gitaka J et al. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



### Ernest Wandera Apondi

<sup>1</sup> Kenya Research Station, Institute of Tropical Medicine, KEMRI/Nagasaki University, Nairobi, Kenya

<sup>2</sup> Directorate of Research and Innovation, Mount Kenya University, Thika, Kenya

### Jesse Gitaka

<sup>1</sup> School of Medicine, Mount Kenya University, Thika, Kenya

<sup>2</sup> Directorate of Research and Innovation, Mount Kenya University, Thika, Kenya

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.**

Author Response 19 Oct 2020

**Arnold Wasike Lambisia**, Kenya Medical Research Institute-Wellcome Trust Research Programme, Centre for Geographic Medicine Research, Kilifi, Kenya

We thank the handling editor and the peer reviewer for the very important comments and suggestions received. We have revised the manuscript in light of this feedback. Below we provide a point-by-point response to the queries raised by the reviewer.

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.

We restrained from calculating pooled testing protocol sensitivity and specificity since that required having results on a minimum number of samples (after sample size calculation) processed in parallel with a gold standard method which we did not have or include in our study. Plans are underway for analyzing a set of samples in our laboratory with the pooled testing protocol and the standard individual testing to provide data on the sensitivity and specificity of the protocol and this will be presented in our follow up analysis.

1. 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.

The significance of missing weak positive cases depends on the reason for testing. For example if it is to identify who is positive so as to put them into isolation to slow down or stop spread, then there is not much loss as weak positives are less likely to contribute to onward transmission. However if the aim is identify how many people have been infected to calculate particular epidemiological parameters e.g. infection prevalence, then there is a danger of their underestimation. Strategies that can improve the sensitivity of the pooled testing protocol include (i) increasing the sample volume during nucleic acid extraction (but this has to be balanced with other extraction kit reagents e.g. the lysis buffer) (ii) using high performance nucleic acid extraction kits and (iii) loading a higher volume of extracted nucleic acid from the pooled samples into the RT-PCR reaction. We have included this discussion into the revised manuscript.

1. Was there any method to the pooling of samples? Was it random or non-random?

Samples collected from a known infected individuals (as per our previous test on the same individual) to check if they were still virus positive and samples from deceased individuals to investigate if they died of COVID-19 were always processed singly. All other samples were processed using the pooled testing protocol. The sample pools were assigned randomly following the consecutive order in which the samples were delivered in the laboratory by the Rapid Response Teams (RRTs). This information has been clarified in the revised manuscript.

1. Were the samples from symptomatic or asymptomatic patients?

The clinical records indicated that these samples were from both asymptomatic individuals (n=1009, 72.1%) and symptomatic individuals (n=54, 3.6%). For 364 samples (24.3%), information on the symptom status of the sampled individuals was unavailable. We have added this information into the revised manuscript.

1. What is the effect of viral loads on pool size and test performance?

When the viral load in a sample is low, a large pool size may lead to a false negative. However, it is not possible to know about the patient viral load before testing. Thus, the pool size has to be optimized as described in the paper and elsewhere (considering assay limit of detection, sensitivity, specificity and infection prevalence) to make both economic and clinical purpose sense.

1. Please correct a few typographical errors especially under the section, "Example pooled testing result in KEMRI-Kilifi laboratory."

These have been corrected.

We thank the handling editor and the peer reviewer for the very important comments and suggestions received. We have revised the manuscript in light of this feedback. We have clarified the symptom status of the individuals we tested, the pooled testing protocol inclusion and exclusion criteria and the implications of the sensitivity of pooled testing protocol on application of the arising results. Below we provide a point-by-point response to the queries raised by the reviewer.

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.

We restrained from calculating pooled testing protocol sensitivity and specificity since that required having results on a minimum number of samples (after sample size calculation) processed in parallel with a gold standard method which we did not have or include in our study. Plans are underway for analyzing a set of samples in our laboratory with the pooled testing protocol and the standard individual testing to provide data on the sensitivity and specificity of the protocol and this will be presented in our follow up analysis.

1. 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.

The significance of missing weak positive cases depends on the reason for testing. For example if it is to identify who is positive so as to put them into isolation to slow down or stop spread, then there is not much loss as weak positives are less likely to contribute to onward transmission. However if the aim is identify how many people have been infected to calculate particular epidemiological parameters e.g. infection prevalence, then there is a danger of their underestimation. Strategies that can improve the sensitivity of the pooled testing protocol include (i) increasing the sample volume during nucleic acid extraction (but this has to be balanced with other extraction kit reagents e.g. the lysis buffer) (ii) using high performance nucleic acid extraction kits and (iii) loading a higher volume of extracted nucleic acid from the pooled samples into the RT-PCR reaction. We have included this discussion into the revised manuscript.

1. Was there any method to the pooling of samples? Was it random or non-random?

Samples collected from a known infected individuals (as per our previous test on the same individual) to check if they were still virus positive and samples from deceased individuals to investigate if they died of COVID-19 were always processed singly. All other samples were processed using the pooled testing protocol. The sample pools were assigned randomly following the consecutive order in which the samples were delivered in the laboratory by the Rapid Response Teams (RRTs). This information has been clarified in the revised

manuscript.

1. Were the samples from symptomatic or asymptomatic patients?

The clinical records indicated that these samples were from both asymptomatic individuals (n=1009, 72.1%) and symptomatic individuals (n=54, 3.6%). For 364 samples (24.3%), information on the symptom status of the sampled individuals was unavailable. We have added this information into the revised manuscript.

1. What is the effect of viral loads on pool size and test performance?

When the viral load in a sample is low, a large pool size may lead to a false negative. However, it is not possible to know about the patient viral load before testing. Thus, the pool size has to be optimized as described in the paper and elsewhere (considering assay limit of detection, sensitivity, specificity and infection prevalence) to make both economic and clinical purpose sense.

1. Please correct a few typographical errors especially under the section, "Example pooled testing result in KEMRI-Kilifi laboratory."

These have been corrected.

**Competing Interests:** No competing interests were disclosed.