Peer Review File

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Reviewer A

Authors utilized computational simulations to optimize nucleic acid testing process for COVID-19. The manuscript is well written and structured.

Comment 1: Just one question, did the scenarios affect the performance of the test? affected the sensitivity, specificity and AUC?

Reply 1: Thank you for your revision. We utilized 2019-nCoV nucleic acid detection kit (fluorescent PCR method) produced by Daan Gene Co. Ltd. for clinical application and scenarios. Sensitivity, specificity and AUC are inherent properties of the test that not affected by any scenarios. We added details about its performance in Page 6 Line 113-118.

In the study, we discussed a potential modeling approach to explore highest throughput by adjusting work schedule or facilities while these resources are usually limited. Performance of the test is certain parameters that could be adjust according to local features.

Changes in the text: We added the following in Methods: “2019-nCoV nucleic acid detection kit (fluorescent PCR method) produced by Daan Gene Co. Ltd. were utilized for laboratory testing. The analytical sensitivity of the kit is 500 copies/mL. In clinical evaluation, the positive coincidence rate of the kit was 97.64%, the negative coincidence rate was 99.71%, and the overall coincidence rate was 98.84%.

Reviewer B

The innovation and novelty of this paper should be affirmed. However, at present, the fastest extraction time of nucleic acid on the market is 16 minutes, and the fastest PCR results can be obtained in one and a half hours according to different reagents.

Specimens do not need to be processed and can be extracted directly. Therefore, it is worth considering whether the efficiency of the model is actually improved.

Comment 1: The authors have not mentioned the specific process of RT-PCR and the validity of the assay used. The author should mention the RT-PCR test kit manufacturer assay details such as the limit of detection.

Reply 1: Thank for your revision. We utilized 2019-nCoV nucleic acid detection kit (fluorescent PCR method) produced by Daan Gene Co. Ltd.. We mix 17 μL of PCR reaction solution A and 3 μL of PCR reaction solution B in a PCR reaction tube, then add 5 μL of sample into mixture, and next start PCR process to amplify. The analytical sensitivity of the kit is 500 copies/mL. In clinical evaluation, the positive coincidence rate of the kit was 97.64%, the negative coincidence rate was 99.71%, and the overall coincidence rate was 98.84%. We added detailed description about the kit in Page 6 Line 113-118. More details about testing process and characteristics of the kit have been submitted as supplement.

Changes in the text: We added the following in Methods: “2019-nCoV nucleic acid detection kit (fluorescent PCR method) produced by Daan Gene Co. Ltd. were utilized for laboratory testing. The analytical sensitivity of the kit is 500 copies/mL. In clinical evaluation, the positive coincidence rate of the kit was 97.64%, the negative coincidence rate was 99.71%, and the overall coincidence rate was 98.84%.

Comment 2: I would like to know if the research protocols used in this study were reviewed and accepted by an independent committee before initiation. The authors should mention if informed consent from research participants were given.

Reply 2: Indeed, this is not a clinical trial or animal experiment, we believe no ethical issues were involved in the test. The test was consent by FAHGMU and related documents was submitted. The consent informed was added in Page 6 Line 111-112.

Changes in the text: We added the following in Methods: “Our study was approved by FAHGMU.”

Comment 3: From my perspective, the model is built on the premise that the epidemic situation and the number of samples are stable. In case of epidemic outbreak, can the model bear the load? It needs to be considered in the discussion.

Reply 3: Commonly in epidemic outbreak, there is explosive growth in sample size accompanied by insufficient laboratory members and facilities. We built the model precisely aiming to explore the maximum throughput with limited resources during outbreak.

Changes in the text: We added this explanation in Page 15 Line 301-305: “Here we just provided a simulation approach to explore the maximum throughput with limited laboratory members and facilities. We believe this would be helpful during epidemic outbreak where there is explosive growth in sample size and insufficient resources. We suggest any implementation of measures should adjust to local features.”

Reviewer C

In this study, simulation of nucleic acid testing for COVID-19 was performed by using generally accepted modeling technique and program. The approach seems quite novel and interesting, but lacks many important points to be accepted in general.
Comment 1: The purpose of the simulation study is not clear. Is it for shortening the turnaround time, or for increasing throughput, or for decreasing cost? Many laboratories are in different situation, and the problem to be solved should be clarified at first.

Reply 1: Thank you for your questions. The main purpose of the study is to increase throughput. We utilized the total time that specimens spend in the model as the first outcome, because the less time specimens spend in the procedure, the higher throughput is.

Comment 2: This study includes only simulation results through input of laboratory variables and data and subsequent results. For these results acceptable, one or more of the scenario should be validated in real setting.

Reply 2: Thank you very much for your suggestion. We also hope that our model can be verified. Yet, experimental verification involves clinical testing operations and the comparison among different operating scenarios, approval by clinical ethics and management was necessary. In view to the current epidemic prevention policy, there is no way to implement the verification. We sincerely hope that this model can be provided to medical institutions in relatively under-resourced regions or third world countries as a reference when formulating or modifying testing procedures.

Comment 3: In page 8, at line 153. How was the replication performed? It should be specified in detail.

Reply 3: As mentioned in context, in the simulation we could only assign how many specimens entered the model every period, like every half hour, but the amounts of specimens and the precise arriving time of a certain specimen was somehow random. Hence though input parameters stable, the output may be different. We performed 25 replications with fixed input and analyzed the mean and 95% CI of results.

Changes in the text: We have added more detailed description in Page 9 Line 172-178: "In view that specimens entered the model randomly at half-hour arrival rates, the amounts of specimens and the precise arriving time of a certain specimen in the model wereas somehow random, so replications were needed to ensure result validity. We believed a 95% confidence interval (CI) of less than 1% of the mean would make the results persuasive. After preliminary experiments, 95% CI of the total time was less than 1% of the mean in the basic scenario with 25 replications. So, we performed 25 replications and analyzed the mean and 95% CI of results as a final result in every scenario."

Comment 4: In Table 1, the changes in Scenario 4 and 7 are the same.

Reply 4: Scenario 4 was built based on Scenario 3. Compared to the basic scenario, the working schedule of scenario 4 was adjusted, and specimen tubes containing guanidine isothiocyanate was used. Scenario 7 was built based on Scenario 6. Compared to

scenario 4, we assumed infinite nucleic acid extraction systems and RT-PCR instruments were available, and specimen size was accordingly increased.

Comment 5: English proof-reading is required.

Reply 5: Thank you for your advice. Sook-san Wong, who is one of authors in this manuscript, is a native speaker of English. She would help us intensively polish the English.