Pooled Sampling is an Efficient and Economical Strategy for SARS-CoV-2 Detection in Low-Prevalence Areas

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SUMMARY

Background: Corona virus disease 2019 (COVID-19) is a severe acute respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Different pooling testing strategies have been applied for the detection of SARS-CoV-2. However, the discrepancies among different pooling strategies are still to be explored.

Methods: The aim of this study was to evaluate the two pooling strategies of collecting respiratory specimens for the detection of SARS-CoV-2 RNA. Two groups of five-sample pools were prepared to evaluate the impact of sample pooling and pooled sampling on test sensitivity, respectively. Viral RNA of coronavirus was extracted with the automation system. The N and ORF1ab genes of SARS-CoV-2 RNA were detected with real-time reverse-transcription PCR. The turnaround time of SARS-CoV-2 testing was analyzed before and after the implement of pooled sampling.

Results: The pooled sampling displayed advantages in assay sensitivity over the sample pooling. The implementation of pooled sampling significantly shortened the turnaround time of SARS-CoV-2 testing.

Conclusions: The pooled sampling is an efficient and economical strategy for SARS-CoV-2 detection during the periods of high screening demand in low-prevalence areas.


KEY WORDS

SARS-CoV-2, real-time reverse-transcription PCR, pooled sampling, sample pooling, sensitivity, turnaround time

LIST OF ABBREVIATIONS

COVID-19 - Corona virus disease 2019
SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2
N - nucleocapsid protein
ORF1ab - open reading frame 1ab
LOD - lower limit of detection
Pre-PS - pre-pooled-sampling
Post-PS - post-pooled-sampling
RT-PCR - real-time reverse transcription polymerase chain reaction
RT-LAMP - reverse transcription loop-mediated iso-
INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a severe acute respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. According to WHO, COVID-19 has spread to more than 235 countries, areas, and territories so far. As of 25 July 2021, there have been 193,657,725 confirmed cases of COVID-19, including 4,154,660 deaths, reported to WHO [2].

SARS-CoV-2 transmission routes mainly include respiratory droplet transmission and direct contact spread [3]. Population screening of asymptomatic individuals can be used to limit the spread of SARS-CoV-2 [4]. The earlier the infected person is identified and the sooner the intervention measures can be initiated, the greater the possibility to contain and control the spread of the infection. The present methods used for the detection of SARS-CoV-2 include quantitative reverse-transcription polymerase chain reaction (RT-PCR), reverse transcription loop-mediated isothermal amplification (RT-LAMP), and Chip [5-7]. Among them, real-time RT-PCR has become the most widely used method owing to its feasibility, high throughput, and high sensitivity. However, with the relative shortages of detection kits and skilled staff, the individual sampling strategy cannot meet the high demand during the period of large-scale population screening.

The pooling test strategy, previously revealed as efficient in the screening of other infectious diseases such as HIV [8], has been deployed for the early screening of SARS-CoV-2 in some countries [9-11]. There are two sampling strategies utilized in the pooling tests: a) Pooling sampling, referred to pooling swabs at the sampling site [9]; b) Sample pooling, defined as pooling viral transport media after sampling individually [10]. Both strategies, pooling samples before RNA extraction, were preferable as compared to pooling samples at the RNA level [12]. However, it is unclear whether there are some discrepancies between the two strategies of pooling samples before RNA extraction. We here evaluated the two pooling strategies in the detection of SARS-CoV-2.

MATERIALS AND METHODS

Sample collection

Respiratory specimens were collected in viral transport media. For the individual sampling, one sampling swab was collected into one collection container. For the sample pooling, a 200 µL sample was aliquoted from each individual collection container and then combined into a single tube (i.e., for a five-sample pool, the volume of mixture was 1,000 µL). For the pooled sampling, however, five sampling swabs from five different individuals were collected into the same collection tube. All individual or pooled samples were subjected to the further viral RNA extraction. Individual sampling, sample pooling, and pooled sampling are illustrated in Figure 1.

Comparison of sample pooling and pooled sampling

Two groups of five-sample pools were prepared to evaluate the impact of sample pooling and pooled sampling on test sensitivity, respectively. Each group consists of 10 five-sample pools. In detail, 200 µL weak positive control sample and the same volume of samples from four different SARS-CoV-2 negative individuals was combined into one single tube, and the mixture was used as pool specimen to assess the impact of sample pooling on test sensitivity. For pooled sampling, 200 µL of viral transport media from four different SARS-CoV-2 negative individuals was collected into one single tube. After centrifugation at 5,000 g, for 5 minutes, 200 µL weak positive control sample was added into the same tube and the pellet was resuspended and used as pool specimen to assess the impact of pooled sampling on test sensitivity. The pooling strategy will be selected only if more target genes of SARS-CoV-2 can be identified in the pool containing lower levels of weak positive control sample.

Nucleic acid extraction

Viral RNA of coronavirus was extracted with Ittrack-32 system for total viral nucleic acid (Daan, Guangzhou, China). Specimens were incubated at 56°C for 30 minutes before transferring into the rack of Ittrack-32 system. According to manufacturer’s instructions (Daan, Guangzhou, China), 200 µL heat-treated sample and 20 µL protease K solution were added into the loading wells of the rack of Ittrack-32 system. The purified RNAs were collected in 100 µL of elution buffer.

Real-time reverse-transcription PCR

Real-time PCR detection of SARS-CoV-2 RNA was performed according to the instructions of the manufacturer (Bojie, Shanghai, China). A total of 5 µL of RNA extract was transferred into reaction tubes containing 20 µL of PCR reagents. RT was performed at 50°C for 10 minutes; and amplification was performed for 1 cycle of 95°C for 5 minutes and 45 cycles of 95°C for 5 seconds and 55°C for 45 seconds. Finally, cooling was performed and maintained at 40°C until analysis was finished. The lower limit of detection (LOD) of this real-time RT PCR assay was 500 copies/mL as claimed by the manu-
Table 1. Comparison of pooled sampling and sample pooling.

<table>
<thead>
<tr>
<th>Pool combination</th>
<th>Mixing strategy</th>
<th>N</th>
<th>ORF1ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 weak positive control (5,000 copies/mL) and 4 negative samples</td>
<td>Pooled sampling</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>Sample pooling</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>1 weak positive control (500 copies/mL) and 4 negative samples</td>
<td>Pooled sampling</td>
<td>9/10</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>Sample pooling</td>
<td>4/10</td>
<td>3/10</td>
</tr>
</tbody>
</table>

Table 2. Summary of SARS-CoV-2 testing of clinical samples during 18 days of high demand.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-Pooled-Sampling (Jan 21 - Jan 31, 2020)</th>
<th>Post-Pooled-Sampling (Feb 1 - Feb 7, 2020)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of requested tests</td>
<td>15,758</td>
<td>13,543</td>
</tr>
<tr>
<td>No. of average requested tests (/day)</td>
<td>1,432</td>
<td>1,934</td>
</tr>
<tr>
<td>No. of PCR reactions</td>
<td>16,308</td>
<td>4,516</td>
</tr>
<tr>
<td>Median of TAT (min)</td>
<td>530</td>
<td>350 **</td>
</tr>
<tr>
<td>25% percentile of TAT (min)</td>
<td>358</td>
<td>270</td>
</tr>
<tr>
<td>75% percentile of TAT (min)</td>
<td>769</td>
<td>458</td>
</tr>
</tbody>
</table>

** - p < 0.0001.

Figure 1. The illustration chart for different sampling strategies.

“1 into 1” means one swab was collected into one collection container. “5 to 1” means 200 μL sample was aspirated from each individual collection container and then was added into one single tube. “5 into 1” means five swabs were collected into one collection tube.
Figure 2. The turnaround time of SARS-CoV-2 RNA detection.

Pre-Pooled-Sampling (Pre-PS); Post-Pooled-Sampling (Post-PS). More samples were tested and reported within shorter turnaround time owing to the implementation of pooled sampling.

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**Statistical analysis**

Mann-Whitney test was used to analyze the difference of turnaround time (TAT) of SARS-CoV-2 testing between Pre-Pooled-Sampling and Post-Pooled-Sampling.

**RESULTS**

**Assessment of impact of sample pooling and pooled sampling on assay sensitivity**

The impact of sample pooling and pooled sampling on test sensitivity was evaluated by combining one weak positive control with four negative samples. The pooled sampling displayed advantages in assay sensitivity over the sample pooling since lower levels of target genes of SARS-CoV-2 can be detected in the former derived pool. So, the pooled sampling strategy was selected to collect clinical samples during periods of high demand for SARS-CoV-2 testing. A comparison of pooled sampling and sample pooling was displayed in Table 1.

**SARS-CoV-2 testing of clinical samples**

A total of 29,301 samples were tested over an 18-day high demand period. The total number of tests was 15,758 within 11 days before the implement of pooled sampling and 13,543 within 7 days after the implement of pooled sampling. The implementation of pooled sampling significantly improved the turnaround time of SARS-CoV-2 testing (Table 2 and Figure 2).

**DISCUSSION**

Early identification of SARS-CoV-2-positive patients is an essential measure to cutoff community transmission of SARS-CoV-2. However, large-scale screening demand of SARS-CoV-2 in some low-prevalence areas caused a relative shortage of testing kits, consumables, and skilled staff. Pooled testing is one potential strategy to preserve resources while enhancing the testing capacities and obtaining results earlier. Pooling can be performed in different sites or at different levels. There are two different pooling strategies: sample pooling or pooled sampling. The sample pooling does not save collection tubes since it needs to pool viral transport media (VTM) from five or ten collection tubes in the testing.
lab. However, pooled sampling can preserve collection tubes because it collects five or ten swabs in the same tube at the collection site. Recently published studies had described the high efficacy of sample pooling strategy for COVID-19.

In the present study, we performed the sensitivity tests to evaluate the sensitivity differences between pooled sampling and sample pooling samples. As shown in Table 1, the pooled sampling strategy detected more samples with a concentration of close to the lower limit of detection. The results showed that pooled sampling had advantages over sample pooling in terms of sensitivity in the detection of SARS-CoV-2. In a pilot study, Wang et al. [13] had described that the swab pooling strategy outperformed the VTM pooling strategy when pooling individual samples with low viral load. However, the pooling of samples might reduce the test sensitivity because of pooling dilution [10]. The dilution in the sample pooling resulted in a median loss of 2.87 Ct for E gene, 3.36 Ct for RdRP gene, and 2.99 Ct for N gene although the VTM pooling strategy for SARS-CoV-2 RNA testing are highly efficient across different RNA extraction and amplification platforms [10]. Notably, when a low concentration of SARS-CoV-2 virus is diluted into a pool of negative samples there will be a risk that levels are close to the limit of detection, thus increasing false-negative rates [14].

Moreover, the optimal pool sizes should be estimated to ensure maximum throughput and decrease false-negative rates [15]. There were several prediction models to optimize the pool sizes [16,17]. A simple mathematical model proposed by Aragon-Cacqueo D et al. [16] revealed optimum group numbers that range from eleven to three subjects, depending on the individual prevalence. Another model showed that when the pooling samples were based on age groups, there was a decrease in the number of tests per subject needed to diagnose one subject [17].

In this work, we utilized “5 into 1” pooled sampling strategy (Figure 1) to collect respiratory specimens to detect SARS-CoV-2 RNA. The median TATs for SARS-CoV-2 testing of clinical samples before and after pooled sampling were 530 minutes, and 350 minutes, respectively. The results showed that the implementation of pooled sampling significantly shortened the turnaround time of SARS-CoV-2 testing (Figure 2). The rapid TAT allowed physicians to rapidly rule in or rule out the diagnosis of COVID-19 and provided the local institution for disease prevention real-time information of COVID-19 infections.

In summary, the pooled sampling strategy had better sensitivity than the sample pooling strategy for SARS-CoV-2 RNA testing in which the same extraction and amplification reagents used. The implementation of five-into-one pooled sampling saves resources, improves throughput, and shortens turnaround time in large-scale screening of SARS-CoV-2. The pooled sampling is an efficient and economical strategy for SARS-CoV-2 detection during the periods of high screening demand in low-prevalence areas.

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Ethics Approval and Consent to Participate:

This study was approved by the Medical Ethics Committee of Shenzhen University General Hospital. (IEC No. KYLL-H-2021-0010-01).

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Declaration of Interest:

All authors declared no conflict of interest.

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