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Real-world Evaluation of a Sample Pooling Strategy for Large-Scale Rapid COVID-19 Testing

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ABSTRACT

Background: The worldwide outbreak of COVID-19 has become a public health crisis of unprecedented proportions. The fast spread of emerging variants increases the needs of rapid diagnostic and screening testing. Sample pooling efficiently expands the testing capacity under limited resources.

Objectives: We evaluated the performance of sample pooling on the Point-of-Care (POC) Liat® and cobas® 6800 systems and provided real-world experiences for implementing these systems in large-scale screenings.

Methods: Positive nasopharyngeal (NP) specimens with Ct values < 25, 25–30 or > 30 were tested individually and in pools to optimize the POC Liat® and cobas® 6800 systems, which were then implemented in community screenings.

Results: The 5-sample pooling strategy did not affect the positive detection rates on Liat® or cobas® 6800 in samples with Ct values < 25 or 25–30. However, in samples with low viral loads (Ct values > 30), five-sample pooling has a higher positive detection rate on POC Liat® (20/20; 100%), compared to cobas® 6800 (9/20; 45%). Five-sample pooled on POC Liat® and two-sample pooled on cobas® 6800 appear to be appropriate for SARS-CoV-2 detection. By implementing the pooling strategies in two large-scale community screenings, 7,606 NP specimens was tested within 36 h; the average turn-around time was 4.8 h for cobas® 6800 and 1.3 h for POC Liat®. Eight positive specimens (0.11%; 8/7,606) were identified, with Ct values ranging from 18.85 to 37.68.

Conclusion: The performance of sample pooling on POC Liat® was demonstrated to be an effective, accurate, and economical approach for large-scale community screenings for COVID-19.

Introduction

The Coronavirus Disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread rapidly worldwide since December 2019 [1–3]. As the pandemic goes on, the emergence of new SARS-CoV-2 variants with increased transmissibility, including the latest Omicron strain, has resulted in multiple worldwide waves of COVID-19; therefore, SARS-CoV-2 testing is in high demand to early identify the infectious patients. However, the SARS-CoV-2 RT-PCR assays, the gold standard for identifying individuals who are currently infected [4,5], generally involve batch testing and may require several hours from sample collection to result reporting [6,7]. The point-of-care (POC) diagnostics of SARS-CoV-2 with a turn-around-time (TAT) of less than 30 min are now available for rapid diagnosis [8], however, their application on the large-scale screening is limited.

The sample pooling strategy firstly proposed by economist Robert Dorfman in 1943 for screening syphilis in US soldiers during World War II has become an important tool during the COVID-19 pandemic for large screening of possibly infected patients [9–12]. However, there are concerns regarding the sample pooling strategy. First, pooling multiple samples into a single reaction may result in diluting weakly positive specimens into mixtures of negative specimens and lead to potential detection failure and false negativity. Second, the sample pooling workflow, requiring considerable hands-on time in preparing samples into different pools and assigning the pool results into individual reports, may lead to a decrease in testing efficiency [13].

The U.S. FDA has authorized the use of sample pooling to the cobas® SARS-CoV-2 test using cobas® 6800/8800. However, studies on the application of sample pooling to POC Diagnostics for SARS-CoV-2
remain limited. In this study, we applied the sample pooling strategy to the Liat® and cobas® 6800 systems and provided real-world experiences for implementing the strategy in large-scale community screenings during the recent resurgence of COVID-19 infection in Taiwan.

Materials and methods

Nasopharyngeal swab samples obtained from Taipei Veterans General Hospital between March and July 2021 were collected in universal transport media (UTM) (Copan, Murrieta, CA) for SARS-CoV-2 nucleic acid testing. For cobas® 6800, a 350 μl aliquot of each sample was mixed with 350 μl MagNA Pure 96 External Lysis Buffer for inactivation of the viral particles. After centrifugation, the samples were loaded onto the Roche cobas® 6800 system, which is a dual-target RT-PCR assay targeting the ORF1a/b and E genes. Samples are considered positive if ORF1a/b or E or ORF1a/b show positive signals; negative if both targets show negative signals; equivocal if only E show positive signals.

The Liat® is a Point-of-Care molecular platform automating the RT-PCR processes including sample extraction/amplification/detection/reporting in a rapid manner for 20 min. The cobas® SARS-CoV-2 & Influenza A/B assay is a multiplex RT-PCR assay run on the Liat® platform for rapid-discrimination of SARS-CoV-2, influenza A and influenza B viruses [14]. For SARS-CoV-2, the test utilizes a dual-target but one-channel detection design for detecting the ORF1a/b and N genes simultaneously. Samples are considered SARS-CoV-2 positive if either or both viral targets give positive signals. The TaqPath COVID-19 Combo Assay (TaqPath) (Thermo fisher scientific inc.) targeting the ORF1ab, N and S genes was used as a confirmatory assay.

The limit of detection (LoD) of cobas® 6800 and Liat® systems was assessed by using the inactivated and quantitated SARS-CoV-2 standard obtained from Taiwan Food and Drug Administration (Lot number 109–06, 1.26E+06 CCID50/mL). The quantitated SARS-CoV-2 standard was serially diluted and tested with 5 replicates. The experimental LoD was defined as the lowest concentration with a detection rate of 100%, and the Probit LoD was estimated at 95% detection rate using probit analysis by SPSS v22.0.

For sample pooling on the cobas® 6800 system, two to five individual samples were pooled into a total volume of 400 μl, and after mixing with 400 μl lysis buffer, the pooled samples were subjected to the cobas® 6800 system for SARS-CoV-2 testing. For sample pooling on the Liat® system, an equal amount of five individual samples was pooled to a total volume of 400 μl and then transferred to Liat® cartridges for analysis.

To increase the working efficiency and reduce manual errors, we implemented a pooling program in the laboratory informatics system (LIS) by which the individual patient samples within a pool are automatically associated with a unique pooling barcode during sample reception. The pooling barcode was recognized by the cobas® 6800 and Liat® systems. After analysis, if the result of the pool is negative, the “negative” result corresponding to the individual patient samples is automatically reported; if the result of the pool is positive, there will be a flag on the LIS to suspend the reporting and further deconvolution will be requested to identify positive samples.

The study was approved by the Institutional Review Board of Taipei Veterans General Hospital.

Results

A total of 46 nasopharyngeal specimens were used for comparing the performance between the Liat® and cobas® 6800 systems, of which 26 were detected as positive and 20 as negative using the Liat® system, while 16 as positive, 10 as equivocal (Ct values of the E gene over 35 on cobas® 6800 but less than 35 on Liat®) and 20 as negative using the cobas® 6800 system (Table 1). The 10 equivocal cases were further confirmed to be positive by the TaqPath assay (Table 2).

To evaluate the detection sensitivity of Liat® and cobas® 6800 systems, the SARS-CoV-2 standard with known viral concentration was serially diluted and subjected to LoD analysis (Supplemental Table S1). The Ct values of tests with varied viral dilutions on the Liat® and cobas® 6800 systems were shown in Supplemental Figure S1. These results showed that the LoD toward SARS-CoV-2 detection of Liat® is lower than that of cobas® 6800.

We then compared the performance of cobas® 6800 and Liat® systems on 5-sample pooling strategy. A total of 60 positive specimens, in which 20 having Ct values < 25 (group 1), 20 having Ct values 25–30 (group 2), and 20 having Ct values > 30 (group 3) determined by the cobas® 6800 system, were individually mixed with 4 negative specimens and analyzed using the Liat® and cobas® 6800 systems. These samples were also individually subjected to Liat® analysis. As shown in Fig. 1 and Supplemental Table S2, in cobas® 6800, the median Ct values of ORF1ab/E genes in group 1 were 20.29/20.12 for individual and 24.31/24.38 for pooled with the mean of the Ct value differences between pooled and individual (ΔCt) of 3.62/3.89; in group 2, those were 26.98/27.07 for individual and 29.76/30.50 for pooled with the ΔCt of 2.83/2.72; in group 3, those were 33.60/35.38 for individual and 31.99/35.03 for pooled (positive pools) with the ΔCt of 0.34/0.93. In Liat®, the median Ct values in group 1 were 14.14 for individual and 16.65 for pooled and the ΔCt was 2.02; in group 2, those were 21.48 for individual and 23.70 for pooled and the ΔCt was 2.19; in group 3, those were 29.50 for individual and 31.57 for pooled and the ΔCt was 2.05. The positive rates were 100% (40/40) detected in the pools of groups 1 and 2 using either Liat® or cobas® 6800 systems. However, in pools of group 3, the positive rate was 45% (9/20) in

| Table 1 SARS-CoV-2 assay comparison between cobas® 6800 and POC Liat® systems (N = 46). |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Assay** | **cobas® 6800 (E and Orf1ab)** | | | | |
| | **Result** | **Positive** | **Equivocal** | **Negative** | **Total** |
| Liat® (N and Orf1ab) | Positive | 16 | 10 | 0 | 26 |
| | Negative | 0 | 0 | 20 | 20 |
| | **Total** | 16 | 10 | 20 | 46 |

| Table 2 Results of the POC Liat® and TaqPath COVID-19 Combo assays of equivocal cases from cobas® 6800 testing. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **No.** | **cobas® 6800** | **Ct of Orf1ab** | **Ct of E** | **Liat®** | **Ct of N/Orf1ab** | **Interpretation** | **TaqPath COVID-19 Combo kit** | **Ct of Orf1ab** | **Ct of S** | **Ct of N** | **Interpretation** |
| 1 | N/D | 38.71 | 31.18 | Positive | 34.25 | 33.89 | 33.78 | Positive |
| 2 | N/D | 38.32 | 32.05 | Positive | 36.75 | 39.42 | 35.42 | Positive |
| 3 | N/D | 37.01 | 32.31 | Positive | N/D | 36.43 | 36.15 | Positive |
| 4 | N/D | 38.60 | 34.34 | Positive | 36.34 | 38.14 | 36.73 | Positive |
| 5 | N/D | 37.51 | 29.53 | Positive | 32.62 | 34.34 | 35.33 | Positive |
| 6 | N/D | 38.13 | 28.62 | Positive | 34.42 | 34.55 | 33.77 | Positive |
| 7 | N/D | 37.73 | 30.57 | Positive | 34.72 | 34.93 | 34.28 | Positive |
| 8 | N/D | 39.23 | 30.91 | Positive | 35.19 | N/D | 35.32 | Positive |
| 9 | N/D | 39.19 | 31.09 | Positive | 35.53 | N/D | 35.04 | Positive |
| 10 | N/D | 37.50 | 27.70 | Positive | 31.14 | 32.23 | 32.19 | Positive |
Fig. 1. Distribution of the Ct values of samples with or without 5-sample pooling.

The Ct values with or without 5-sample pooling on cobas® 6800 and POC Liat® systems were presented. The samples were classified into three groups, group 1 with Ct values < 25, group 2 with Ct values ranging 25–30, and group 3 with Ct values > 30, according to their individual Ct values obtained from the cobas® 6800 system. (A) Ct values of Orf1ab on the cobas® 6800, (B) Ct values of E on the cobas® 6800, (C) Ct values of N/Orf1ab on the POC Liat®. Pools with Ct values more than 40 were automatically interpreted as “N/Do” (not detected) and were not included in the figure. Error bars indicate mean ± SD of individual values from the mean.

cobas® 6800 and 100% (20/20) in Liat® (Table 3 and Supplemental Table S2). These findings demonstrated that the 5-sample pooling strategy did not affect the positive detection rate on Liat® but had a compromise on cobas® 6800, especially in samples with low viral loads (Ct > 30).

To further validate the performance of the 5-sample pooling strategy on the Liat® system, 363 nasopharyngeal specimens, including 12 positive and 351 negative cases, were analyzed. As shown in Table 4, by comparing pooling and individual on the Liat® system, the positive percent agreement (PPA) and negative percent agreement (NPA) were both 100%. However, when the results of Liat® pooled were compared to those of individual testing on cobas® 6800, 41.6% (5/12) showed equivocal results, which were further confirmed as weakly positive using the TaqPath assay. Additionally, one follow-up sample obtained from a confirmed COVID-19 patient showing discrepant results (POC Liat®+/+ cobas® 6800−) was also confirmed by the TaqPath assay to be positive, with a Ct value of 34.2 in N gene and 34.7 in ORf1ab gene. The PPA and NPA were 100% (11/11) and 99.7% (351/352), respectively, between Liat® pooled and cobas® 6800 individual. To further optimize the pooling condition on cobas® 6800, 20 positive specimens with the original Ct > 30 obtained from cobas® 6800 were tested (Table 5). In 2-sample pooling, 55% (11/20) of pools were positive, and 45% (9/20) were equivocal. In 3-sample pooling, 35% (7/20) of pools were positive, 35% (7/20) were equivocal, and 30% (6/20) were false-negative. In 4-sample pooling, 30% (6/20) of pools were positive, 35% (7/20) were equivocal, and 35% (7/20) were false-negative. To reduce the false-negative rates, we considered the 2-sample pooling strategy to be viable for cobas® 6800 in detecting SARS-CoV-2.

After successfully holding COVID-19 at bay since 2020, Taiwan, in early May 2021, experienced a COVID-19 outbreak starting with China Airline, Novotel clusters and subsequently spreading to tea parlors in Wanhua. Consequently, there were hundreds of new confirmed cases daily. To effectively prevent disease transmission in the community, in June 2021, expanded SARS-CoV-2 testing was first conducted at an electronics company in Miaoli and then at Binjiang Market in Taipei. The 2-sample pooling strategy run on the cobas® 6800 system and the 5-sample pooling strategy run on the Liat® system were carried out. There were 4948 nasopharyngeal samples, corresponding to 360 Liat® pools (1796 samples) and 1576 cobas® 6800 pools (3152 samples), screened within 24 h for the electronics company, and 2658 nasopharyngeal (1312 samples), screened within 12 h for the Binjiang Market. The samples were delivered from the sample procuring setting. Therefore, pools with less than 5 samples may occasionally occur in performing the POC Liat® during the large community screenings. There were 8 pools, 6 from Liat® pools and 2 from cobas® 6800 pools, showing positive signals. After deconvolution, 8 out of 7606 specimens had SARS-CoV-2 detected with a positivity rate of 0.11% (8/7606). Among the 8 positive cases, 3 showed only E gene positivity with Ct values over 37 using the cobas® 6800 system, and they were further confirmed as positive using the Liat® system. The TAT from sample reception to reporting was 1.3 h for the Liat® system and 4.8 h for the cobas® 6800 system (Fig. 2).

Discussion

In this study, we demonstrated that the sample pooling strategy significantly increases the testing capacity of SARS-CoV-2, in which a total of 7606 tests with an average TAT of 3 h (4.8 h for cobas® 6800 and 1.3 h for Liat®) from sample reception to reporting were completed within 36 h. This is also the first study to evaluate the sample pooling on the Liat® system, demonstrating that it could serve as an effective, accurate, and economical approach for large-community screenings.
The LoD assessment on the Liat® and cobas® 6800 systems showed that the LoD of Liat® is lower than that of cobas® 6800 (Supplemental Table S1). By comparing these two systems, as shown in Table 2 and Table 4, our data suggest that the detection sensitivity of Liat® toward SARS-CoV-2 may be higher than that of cobas® 6800. However, without head-to-head clinical trials, it is immature to make assumptions about relative sensitivity between these two systems. In Table 3, we found the false negative rates among samples with Ct > 30 was 40% (8/20) on cobas® 6800, but no false negatives were identified on Liat®, indicating with 5-sample pooling, Liat®, compared to cobas® 6800, has a higher positive detection rate for samples with low viral loads.

The advantages of using Liat® for pooling include its lower LoD than cobas® 6800 and it is more economical. For individual testing, the reagent cost is around US$ 60/sample for Liat®, US$ 24/sample for cobas® 6800, US$ 24/sample for TaqPath assay, and US$ 12/sample for the antigen test. The cost of reagents and instruments (quoted by the local distributors) regarding sample pooling used in this study were provided in Supplemental Table S3. The application of sample pooling will reduce the PCR reagent cost to be comparable to that of an antigen test. In addition, the testing capacity for Liat® (5-sample pooling; 10 Liat® machines) was 150 tests/hr with 20 min/run, but for cobas® 6800 (2-sample pooling; 2 cobas® 6800 machines) was 376 tests/3 hrs with 3 hrs/run.

Although the US FDA has granted EUA to the cobas® SARS-CoV-2 test on cobas® 6800/8800 systems with pooled specimens of up to six in a single pool, in our study, the appropriate number of pools on cobas® 6800 is two. This discrepancy is most likely due to that in Taiwan, we usually add an external lysis buffer in a 1:1 ratio for virus inactivation, which may result in a twofold dilution. In this study, by assessing the LoD, we found the use of external lysis in cobas® 6800 may slightly

### Table 3
Comparison of the positive detection rate of the 5-sample pooling strategy on cobas® 6800 and POC Liat®.

| Ct group | Results | cobas® 6800 | Liat® |
|----------|---------|-------------|-------|
|          | Individual | Pooled No | Individual | Pooled No |
| Group 1 (Ct<25) | Positive 20 100 | 20 100 | 20 100 | 20 100 |
|          | Negative 0 0 | 0 0 | 0 0 | 0 0 |
|          | Equivocal 0 0 | 0 0 | 0 0 | 0 0 |
|          | Total 20 100 | 20 100 | 20 100 | 20 100 |
| Group 2 (Ct 25–30) | Positive 20 100 | 20 100 | 20 100 | 20 100 |
|          | Negative 0 0 | 0 0 | 0 0 | 0 0 |
|          | Equivocal 0 0 | 0 0 | 0 0 | 0 0 |
|          | Total 20 100 | 20 100 | 20 100 | 20 100 |
| Group 3 (Ct > 30) | Positive 20 100 | 9 45 | 20 100 | 20 100 |
|          | Negative 0 0 | 8 40 | 0 0 | 0 0 |
|          | Equivocal 0 0 | 3 15 | 0 0 | 0 0 |
|          | Total 20 100 | 20 100 | 20 100 | 20 100 |

### Table 4
The performance of sample pooling on the POC Liat® system with reference based on the cobas® 6800 results (N = 363).

| Liat® (Pooled) | Positive (No.) | Negative (No.) | Total (No.) | Positive (No.) | Equivocal (No.) | Negative (No.) | Total (No.) |
|---------------|----------------|----------------|-------------|----------------|----------------|----------------|-------------|
| Individual    | 12             | 0              | 12          | 6              | 5              | 1              | 12          |
| Pooled        | 351            | 351            | 363         | 352            | 363            |

### Table 5
The effect of pool size on the detection of SARS-CoV-2 in samples with Ct values over 30.

| Sample No. | Individual Orf1ab E | 2-sample pooled Orf1ab E | 3-sample pooled Orf1ab E | 4-sample pooled Orf1ab E |
|------------|---------------------|--------------------------|--------------------------|--------------------------|
| 1          | 31.37               | 30.10                    | 31.18                    | 31.08                    |
| 2          | 31.44               | 32.08                    | 32.27                    | 32.27                    |
| 3          | 33.76               | 37.31                    | N/D                      | 35.84                    |
| 4          | 34.98               | 36.44                    | N/D                      | 36.27                    |
| 5          | 35.56               | 37.20                    | N/D                      | 35.52                    |
| 6          | 32.92               | 35.51                    | N/D                      | 36.99                    |
| 7          | 30.74               | 31.16                    | 31.39                    | 33.78                    |
| 8          | 33.86               | 37.92                    | N/D                      | 34.79                    |
| 9          | 34.05               | 36.33                    | N/D                      | 36.94                    |
| 10         | 30.34               | 30.84                    | 31.08                    | 31.72                    |
| 11         | 34.64               | 36.26                    | N/D                      | 36.17                    |
| 12         | 33.83               | 33.69                    | 37.40                    | 37.97                    |
| 13         | 31.50               | 31.15                    | 32.24                    | 31.93                    |
| 14         | 35.07               | 39.14                    | N/D                      | 35.54                    |
| 15         | 34.50               | 36.15                    | N/D                      | 36.17                    |
| 16         | 35.56               | 34.01                    | 36.88                    | 34.41                    |
| 17         | 30.79               | 32.04                    | 32.93                    | 31.19                    |
| 18         | 33.69               | 33.78                    | N/D                      | 34.03                    |
| 19         | 37.35               | 37.82                    | N/D                      | 34.48                    |
| 20         | 33.51               | 33.59                    | 35.95                    | 32.40                    |

N/D: not detected.
impair the assay LoD (Supplemental Table S1).

The use of pooling in large-community screenings could effectively increase testing capacity and preserve testing reagents/resources. However, in high prevalence situations, e.g., when 5-sample pooling is used in the areas with a prevalence > 25%, it may become less efficient and lead to delayed test results since positive pools will need to be deconvoluted. The efficiencies of pooling samples take place when most pools test negative, a scenario highly related to the infection prevalence. Kim et al. has reported that the probability of a negative pool ($p$) is given by $\theta = (1 - p)^s$ for a prevalence ($p$) and pool size ($s$) [15]. Thus, before executing sample pooling, laboratories should estimate the prevalence to determine the optimal pool size for performing testing most efficiently.

The difference in pooling methods may have various impacts on the detection sensitivity [16]. The VTM pooling method usually leads to a decrease in viral loads in the pooled samples due to sample dilution, which may increase the risk of false negatives [17,18]. On the contrary, the swab pooling method was found to be as sensitive as individual testing except for the RNA pooling method allows no need for the repeated sample collection or RNA extraction during positive deconvolution, it cannot be applied in some automation systems that incorporate the RNA extraction and the real-time PCR in a closed and fully-automated workflow, such as the cobas® 6800 system.

Sample pooling also increases the complexity of laboratory procedures relative to individual testing, leading to an increased risk of cross-contamination. To provide reliable results, adequate automation workflows such as the implementation of pooling robotic machines and/or the LIS for assisting sample processing and reporting are important. In this study, instead of using the automatic pooling bots, we have successfully adapted the LIS to assist the sample pooling procedures.

Collectively, we demonstrated the application of sample pooling on cobas® and Liat® are robust methods for large-scale screening for COVID-19, which is helpful for the control of community transmission of SARS-CoV-2.

**Declaration of Competing Interest**

The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2022.105133.

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