SHORT COMMUNICATION

Sample Pooling: A Cost-Saving Strategy for SARS-CoV-2 Screening in Hospitalized Patients and Attending Relatives – Insights from a Secondary Care Hospital Amidst the Omicron Wave

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Background: Pooled real-time reverse transcriptase polymerase chain reaction (rRT-PCR) for SARS-CoV-2 screening is cost- and labor-saving, but data during the Omicron wave are lacking.

Objective: To examine a correlation between pooled rRT-PCR as a screening method for SARS-CoV-2 and community COVID-19 incidence.

Materials and Methods: The authors retrospectively collected data on pooled rRT-PCR testing as a COVID-19 screening strategy between February 1 and April 30, 2022 in the present study center.

Results: In comparison to performing single rRT-PCR testing, pooled rRT-PCR testing strategy reduced the numbers of rRT-PCR testing by 73.36%, 53.63%, and 54.7% in February, March, and April, respectively. The community COVID-19 incidence rate per day (CCIR) averaged 25.43, 48.12, and 80.96 per 100,000 population per day during February, March, and April, respectively.

Conclusion: Small pooled rRT-PCR testing of up to five samples may be a cost-effective screening strategy among selected populations despite high CCIR during the Omicron wave.

Keywords: Pooled rRT-PCR; Screening

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To prevent the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), one must understand the principal route of transmission. Airborne and droplet transmissions remain the principal routes. Therefore, mask wearing is essential to prevent in-hospital transmission of SARS-CoV-2[1]. However, compliance with mask wearing among patients visiting hospitals was reported to be suboptimal at 78%[2]. An additional screening strategy for SARS-CoV-2 should also be implemented during the global spread of B.1.1.529 (Omicron) and its subvariants with high transmissibility[3,4]. Real-time reverse transcriptase (rRT-PCR) is considered the gold standard for diagnosing SARS-CoV-2 infection, but its cost-effectiveness weighs in routine use. The pooling of samples for PCR testing demonstrated similar sensitivity and specificity compared with single rRT-PCR testing using a small pool size of five samples[5].

In the present study, the authors demonstrated the real-life use of a pooling strategy for SARS-CoV-2 screening during the Omicron variant pandemic. The authors retrospectively collected data on pooled samples between February 1 and April 30, 2022 in a 109-bed secondary care center, Suddhavej Hospital, Mahasarakham University, Mahasarakham, Thailand. Isolated VIP rooms accounted for 35 beds, otherwise, patients were admitted to one of four general wards

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or 5-bed intensive care units (ICUs) with a 2-meter distance between each bed, with one airborne isolation room in the ICU. Either single or pooled rRT-PCR testing was required in all hospitalized patients in the present study center. Pooling strategy was performed in 1) attending relatives, 2) self-paying outpatient individuals, 3) planned elective surgery, and 4) hospitalized patients except for the following, emergency or urgent operations and patient under investigation (PUI) for coronavirus disease 2019 (COVID-19). PUI for COVID-19 included 1) clinically compatible with COVID-19, including any or combined fever, all upper and lower respiratory symptoms, hyposmia or anosmia, dyspnea, or desaturation, 2) fever with unidentified source and recent exposure to SARS-CoV-2 within 14 days, 3) unexplained pneumonia, and 4) clinically suspicious of COVID-19 per the physician's discretion.

Each pool consisted of five nasopharyngeal samples mixed prior to RNA extraction. Two to five samples per pool might be performed to match the number of samples per day such as 14 samples can be divided into five, five, and four samples in three pools. The authors examined the relationship between the positivity rate of pooled PCR testing each day and the community COVID-19 incidence rate per day (CCIR). Daily reports of COVID-19 cases were counted only if positive rRT-PCR or rapid antigen tests were confirmed. These numbers included data from all 13 subprovincial districts consisting of 953,660 people as of 2020[6]. All confirmed COVID-19 cases during the study period were required to be reported to the Mahasarakham Provincial Public Health Office under the Dangerous Communicable Disease Act 2015. CCIR was reported as cases per 100,000 population per day. Secondary outcomes were hospital-acquired COVID-19 infection among patients during the study period. Hospital-acquired COVID-19 infection was defined as 1) COVID-19 infection detected during admission by rRT-PCR, 2) symptom onset occurring after at least 72 hours of hospitalization, 3) initial negative rRT-PCR testing prior to admission, and 4) not compatible with PUI criteria at the first day of admission. The present study was approved by the Ethics Committees of Mahasarakham University (No.392-182/2565).

A simple linear regression model was used to formulate a linear equation to predict the positivity rate of pooled rRT-PCR each day from CCIR. The predicted value in the linear regression model was presented with 95% confidence intervals (CIs). The Mann-Whitney U-test was used to calculate the median difference. IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism, version 9 (GraphPad Software, Inc., San Diego, CA, USA) were used for statistical analysis. A p-value of less than 0.05 was statistically significant.

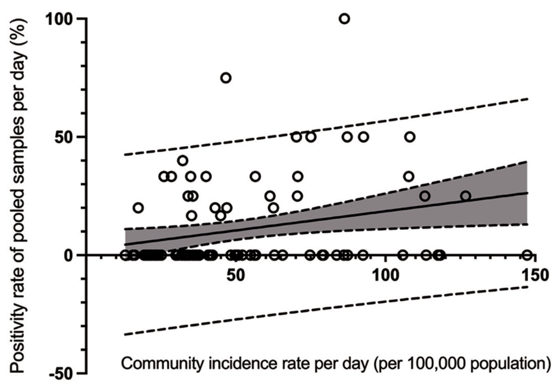

Figure 1. Simple linear regression showed a significant relationship between the community incidence rate and positivity rate of pooled PCR testing (p=0.017) (R^2=0.06375). The gray area represents the 95% CI.

There were 1,412 samples accounting for 342 pooled samples tested (3.84 pools per day). Eight hundred eighty-four (62.61%) were hospitalized patients, 426 were elective surgeries, and 528 (37.39%) were attending relatives or self-paying individuals. Pooled samples were obtained from 62.08% (884/1,424) hospitalized patients, excluding emergent and urgent surgical patients and COVID-19 patients where the total admission was 4,982 days among 1,424 patients. The positivity rate of pooled samples per day correlated linearly with CCIR (Figure 1). The most common non-elective-surgery in-patient diagnoses for pooled testing were thalassemia and chronic kidney disease stage 5 (Figure 2A). The summation of diagnoses that accounted for less than 1% is included in the "other" in Figure 2A.

In comparison to performing a single PCR test, the pooling strategy reduced the number of PCR tests by 73.36%, 53.63%, and 54.7% in February, March, and April, respectively. CCIR averaged 25.43, 48.12, and 80.96 per 100,000 population per day during February, March, and April, respectively. There were 4.39% (15/342) true positive pools and 2.34% (8/342) false positive pools. Median Ct values of *N* and *ORF1ab* genes among false positive pools were indifferent from true positive pools at 32.14 versus 32.74 with a median difference of 0.65 (95% CI –8.780 to 5.14, p>0.999) for *N* gene, and 34.08 versus 33.52 with a median difference of –0.56

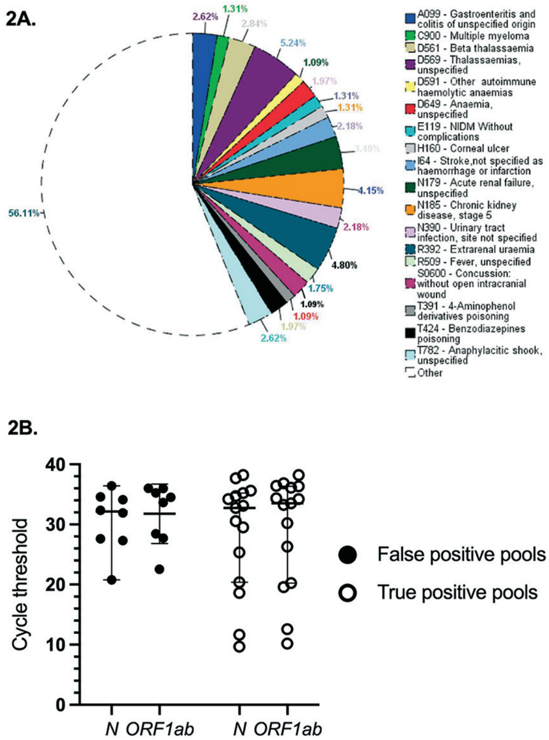

Figure 2. (A) The pie chart demonstrates the proportion of in-patient diagnoses according to the ICD-10. (B) Scatter dot plot demonstrating the Ct value between each target gene in false- and true-positive pools. The thick and thin lines within a scatter dot plot represent the median and 95% CI, respectively.

(95% CI –8.86 to 3.77, p=0.925) for *ORF1ab* gene, respectively (Figure 2B). The authors formulated an equation from a simple linear regression model as follows: Y = 0.1623*X + 2.355, where Y was the percentage of positive pooled PCR per day and X was the CCIR.

There were two hospital-acquired COVID-19 patients, which accounted for <0.001 cases per 1,000 person-days during the present study period. One patient contracted SARS-CoV-2 from an attending relative who occasionally left the hospital and had not been retested for SARS-CoV-2 for 20 days since the first test. The other patient acquired SARS-CoV-2 from an adjacent patient with COVID-19 pneumonia who had prolonged viral shedding.

In conclusion, small pooled rRT-PCR testing of up to five samples may be a cost-effective screening strategy among selected populations despite high CCIR given the gradual slope of the correlation. A cost reduction of up to 75% can be achieved, which is similar with the previous studies(7,8). The present real-life study adds to the pre-existing evidence using a small pool size for hospital surveillance(5). Despite increasing hospital-onset COVID-19 during the Omicron surge worldwide(9,10), combining single and pooled rRT-PCR testing as universal screening for all hospitalized patients contributed to low hospital-acquired COVID-19 during the Omicron wave in the present study center.

What is already known on this topic?

Several modeling studies have suggested that employing pooled rRT-PCR testing can be a cost-effective approach for screening during the COVID-19 pandemic. These studies have proposed small pool sizes, typically up to five samples, for monitoring SARS-CoV-2 infections in hospital settings. However, there is limited real-world evidence demonstrating the safety and cost-effectiveness of this strategy.

What does this study add?

This research offers evidence supporting the use of pooled rRT-PCR testing for SARS-CoV-2 as a screening method among specific hospitalized individuals and attending relatives with a low risk of COVID-19. This approach may prove to be cost-effective and result in a notably low incidence of hospital-acquired COVID-19, particularly amidst the widespread Omicron variant community infections.

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Conflict of interests

The authors declare no conflict of interests.

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