

The Application of Sample Pooling for Mass Screening of SARS-CoV-2 in an Outbreak of COVID-19 in Vietnam

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Abstract. We sampled nasal–pharyngeal throat swabs from 96,123 asymptomatic individuals at risk of SARS-CoV-2 infection, and generated 22,290 pools at collection, each containing samples from two to seven individuals. We detected SARS-CoV-2 in 24 pools, and confirmed the infection in 32 individuals after resampling and testing of 104 samples from positive pools. We completed the testing within 14 days. We would have required 64 days to complete the screening for the same number of individuals if we had based our testing strategy on individual testing. There was no difference in cycle threshold (Ct) values of pooled and individual samples. Thus, compared with individual sample testing, our approach did not compromise PCR sensitivity, but saved 77% of the resources. The present strategy might be applicable in settings, where there are shortages of reagents and the disease prevalence is low, but the demand for testing is high.

SARS-CoV-2 is the cause of the COVID-19 pandemic.¹ According to the WHO, as of December 30, 2020, there have been > 80 million cases and > 1.7 million deaths reported globally.

After the first infection was reported in Vietnam on January 23, 2020,² COVID-19 was brought under control by the second week of April.³ However, after 100 days of no community transmission, on July 25, 2020, SARS-CoV-2 infection was confirmed in a 57-year-old man presenting with pneumonia admitted to C Hospital in Da Nang city, in the center of Vietnam. This was followed by the detection of SARS-CoV-2 in another 61-year-old man who had been mechanically ventilated for 4 days in another hospital of the city (Da Nang hospital).⁴

Rigorous contact tracing and testing detected increased COVID-19 cases in the following week, with most of the cases being linked with Da Nang hospital.⁴ Consequently, the city was locked down on July 28, 2020. In parallel, a mass community testing approach coupled with a novel sample pooling strategy was implemented on the second week of August 2020, contributing to the ultimate success of COVID-19 control in Da Nang city. Indeed, by September 11, 2020, the city experienced lockdown for 12 consecutive days without community transmission.⁴ A total of 389 confirmed cases and 31 deaths were recorded, representing the largest community outbreak of COVID-19 in Vietnam to date. Here, we describe our sample pooling strategy and the results of its application for mass community testing for the period between August 8 and 21, 2020.

In addition to the containment approaches widely applied in Vietnam,⁵ a mass community testing strategy was implemented in Da Nang from the second week of August 2020 onward. Accordingly, this approach was applied to asymptomatic individuals with at least one of the criteria: 1) quarantined individuals with the first SARS-CoV-2 reverse transcription PCR (RT-PCR) negative; 2) visiting Da Nang

hospital between July 15 and 26, 2020, the period with ongoing transmission within the hospital as defined by the local authorities; 3) living in an area where there was a confirmed case of community transmission; 4) a family member of a direct contact of a confirmed case. The testing strategy formed part of the response to the COVID-19 outbreak that was approved by Da Nang Department of Health. Accordingly, obtaining informed consent from individuals was deemed unnecessary.

Because the number of people in a household or the community under investigation may vary, we pragmatically aimed to group two to seven people into one group for testing. We collected a nasal–pharyngeal throat swab (NTS) from each individual of the group, and combined in a single 15-mL collection tube containing 3 mL of viral transport medium (Figure 1A). If SARS-CoV-2 testing of the pool returned positive, we then collected single NTS from each individual of the corresponding group for confirmatory RT-PCR testing (Figure 1A). All collected samples were sent to the laboratory of Da Nang Centre for Disease Control and Prevention which was responsible for 90% of mass screening in Da Nang city. We also collected demographics, contact history, and respiratory signs/symptoms from the confirmed cases using a standard case record form.

We extracted viral RNA from 200 μ L of the pooled NTS samples using Thermo Scientific KingFisher Flex, an Automated Nucleic Acid Extraction Workstation (Thermo Scientific, Waltham, MA), following the manufacturer's instructions. We used LightPower iVASARS-CoV-2 first RT-rPCR kit (Viet A, HCMC, Vietnam) as a screening assay, and the E gene real-time RT-PCR assay (TIB MOLBIOL, Berlin, Germany)⁶ as a confirmatory assay.

For subsequent testing of NTSs obtained from individuals of the corresponding positive pools (Figure 1A), we manually extracted viral RNA from 140 μ L of the NTS samples using the QIAamp viral RNA kit (QIAGEN GmbH, Hilden, Germany), following the manufacturer's instruction, and used the TIB MOLBIOL E gene real-time RT-PCR to detect SARS-CoV-2.

We used Wilcoxon signed-rank test available in GraphPad Prism version 5.04 (GraphPad Software, San Diego, CA) to

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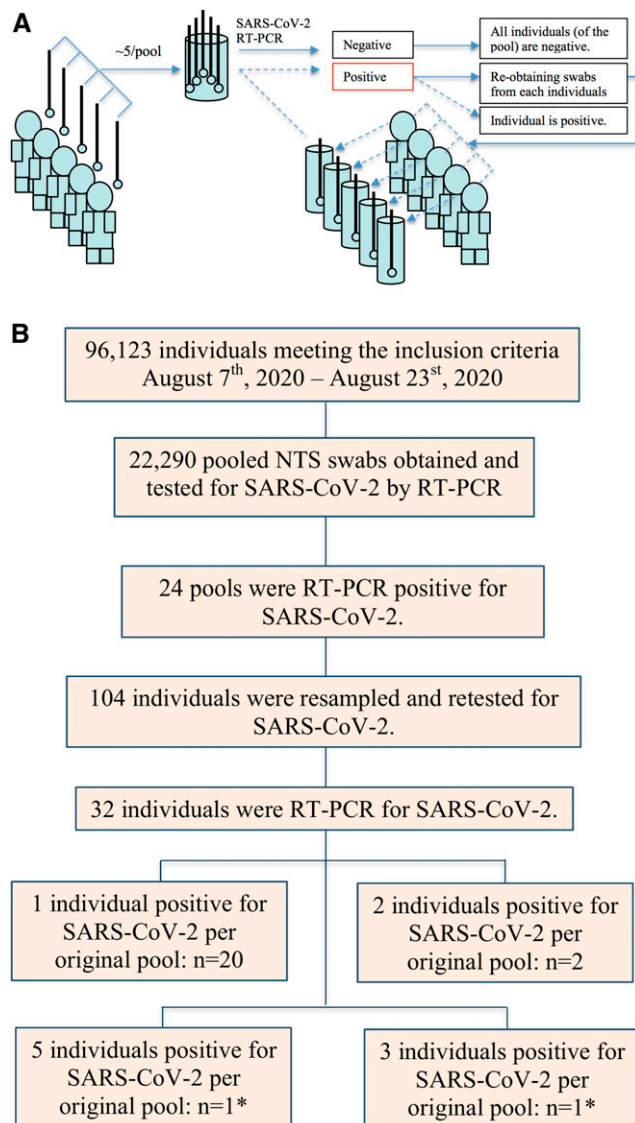


FIGURE 1. An overview about the strategies of sampling and testing of the collected swabs for SARS-CoV-2. (A) Flowchart illustrating the strategy of obtaining the pooled nasal pharyngeal–throat swabs for primary screening of SARS-CoV-2, and follow-up sampling and testing of individuals of the pools who had a positive SARS-CoV-2 RT-PCR result. (B) Flowchart illustrating the mass community testing for SARS-CoV-2 leading to the detection of 32 cases, including eight belonging to two family clusters. Note to (B) *From the same family of five and three people, respectively, who were all positive for SARS-CoV-2, but were all asymptomatic. This figure appears in color at www.ajtmh.org.

compare the Ct values obtained from the pooled samples and individual samples. The Ct values used for the analysis were those generated by the confirmatory assay.

From August 8, 2020 to August 21, 2020, a total of 22,290 pooled NTS samples were collected from 96,123 individuals meeting the inclusion criteria, and successfully tested for SARS-CoV-2. The number of NTSs per pool varied between two and seven, with the majority (58%) having five samples per pool (Supplemental Table 1). The included individuals accounted for 8.2% of the population of 1.1 million in Da Nang city, and came from 7/8 eight districts of the city.

RT-PCR testing revealed evidence of SARS-CoV-2 RNA in 24/22,290 (0.1%) of the pools (Supplemental Table 1). Subsequently, a total of 104 individuals were resampled and tested for SARS-CoV-2 by RT-PCR. Subsequently, 32 individuals belonging to one of the 24 originally positive pools had a confirmed diagnosis of SARS-CoV-2 (Figure 1B), accounting for 21% (32/156) reported cases in Da Nang city during the same period (August 8–22, 2020). There was no difference in Ct values of pooled NTS samples and that of individual NTS samples (Ct values [median, range] for pooled NTS samples: 30.86 [16–37], for individual NTS samples: 30.33 [15.36–34], P comparison = 0.23) (Figure 2).

The time from pooled sample collection to reporting the confirmed diagnostic result was either the same day ($n = 9$), 24 hours ($n = 10$), or 48 hours ($n = 12$). In the remaining cases, the result was released within three working days.

By pooling, we successfully saved 73,833 RT-PCR tests and associated consumables (corresponding to a reduction of 77% of the costs). In our setting, the associated cost to conduct RT-PCR testing for SARS-CoV-2 in one suspected case was ~21.5 United States dollar (USD). Thus, excluding manpower, our mass screening strategies saved ~1.5 million USD. Because our available resources could only accommodate some ~1500 SARS-CoV-2 RT-PCRs per days for mass screening, it would have taken 64 days to complete the screening for those 96,123 individuals compared with 14 days required by our pooling strategy.

The 32 SARS-CoV-2–positive individuals included 21 females and 11 males aging from 14 to 73 years (median 45 years). None had any signs/symptoms of respiratory infection at the time of collection. Of these, 22 had no history of contact with a confirmed COVID-19 case (Table 1, Figure 1B). Two family clusters with all eight members were positive for SARS-CoV-2 and were all asymptomatic. None recalled of having any signs/symptoms of respiratory infections.

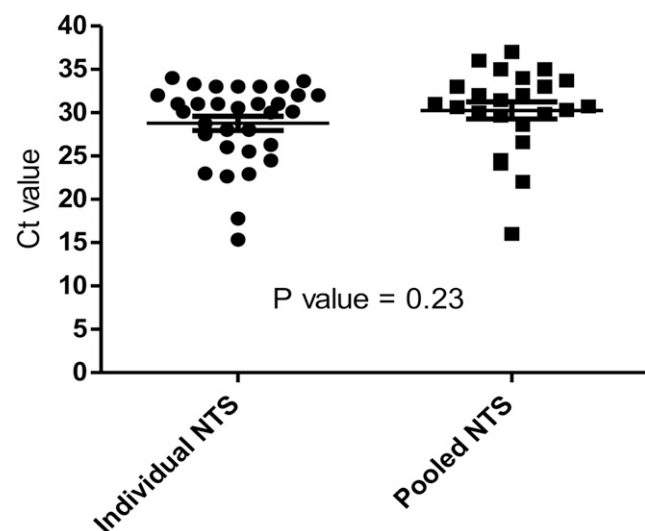


FIGURE 2. Plots showing real-time RT-PCR Ct values of pooled nasal pharyngeal–throat swab (NTS) samples and individual NTSs. A total of 24 pooled NTS samples and 32 individual NTS samples were included for analysis. Ct values (median, range): for pooled NTS samples: 30.86 (16–37), for individual NTS samples: 30.33 (15.36–34). P comparison = 0.23.

TABLE 1
Epidemiological factors of SARS-CoV-2-positive individuals

Patient number*	Reason for being tested	Recalled date of contact	Tested positive on	Risk group†
1	A neighbor of a confirmed case. No known contact with a confirmed case		August 9	1
2	Living in the same apartment buildings of a confirmed case	Not applicable	August 10	1
3	A neighbor of a confirmed case. No known contact with a confirmed case	Not applicable	August 10	1
4	Visited Da Nang hospital at any time point, July 15, 2020–July 26, 2020. No known contact with a confirmed case	Not applicable	August 11	1
5	Visited Da Nang hospital at any time point, July 15, 2020–July 26, 2020. No known contact with a confirmed case	Not applicable	August 11	1
6	No known contact. Living in an area with recorded community transmission	Not applicable	August 12	1
7	Visited Da Nang hospital at any time point, July 15, 2020–July 26, 2020. No known contact with a confirmed case	Not applicable	August 12	1
8	Visited Da Nang hospital at any time point, July 15, 2020–July 26, 2020. No known contact with a confirmed case	Not applicable	August 15	1
9	Visited Da Nang hospital at any time point, July 15, 2020–July 26, 2020. No known contact with a confirmed case	Not applicable	August 15	1
10	Visited Da Nang hospital at any time point, July 15, 2020–July 26, 2020. No known contact with a confirmed case	Not applicable	August 15	1
11	A neighbor of a confirmed case without a known contact	Not applicable	August 15	1
12	Visited Da Nang hospital at any time point, July 15, 2020–July 26, 2020. No known contact with a confirmed case	Not applicable	August 17	1
13	Visited Da Nang hospital at any time point, July 15, 2020–July 26, 2020. No known contact with a confirmed case	Not applicable	August 17	1
14	A businessperson at a market where mass community testing was conducted. No known contact with a confirmed case	Not applicable	August 18	1
15	No known contact. Living in an area with recorded community transmission	Not applicable	August 18	1
16	Visited Da Nang hospital at any time point, July 15, 2020–July 26, 2020. No known contact with a confirmed case	Not applicable	August 20	1
17	Visited Da Nang hospital at any time point, July 15, 2020–July 26, 2020. No known contact with a confirmed case	Not applicable	August 20	1
18	Visited Da Nang hospital at any time point, July 15, 2020–July 26, 2020. No known contact with a confirmed case	Not applicable	August 20	1
19	A businessperson at a market where mass community testing was conducted. No known contact with a confirmed case	Not applicable	August 22	1
20	A businessperson at a market where mass community testing was conducted. No known contact with a confirmed case	Not applicable	August 21	1
21	A businessperson at a market where mass community testing was conducted. No known contact with a confirmed case	Not applicable	August 21	1
22	A guard at a market where mass community testing was conducted. No known contact with a confirmed case	Not applicable	August 22	1
23	A contact of a confirmed case. First SARS-CoV-2 RT-PCR was negative.	July 20, 2020	August 12	2
24	A family member (patient 26) was a contact of a confirmed case.	July 20, 2020 onward	August 12	2
25	A family member (patient 26) was a contact of a confirmed case.	July 20, 2020 onward	August 12	2
26	A family member (patient 26) was a contact of a confirmed case.	July 20, 2020 onward	August 12	2
27	A family member (patient 26) was a contact of a confirmed case.	July 20, 2020 onward	August 12	2
28	A contact of a confirmed case. First SARS-CoV-2 RT-PCR was negative.	July 20, 2020 onward	August 15	2
29	A contact of a confirmed case. First SARS-CoV-2 RT-PCR was negative.	July 24, 2020 onward	August 17	2
30	A contact of two confirmed cases. First SARS-CoV-2 RT-PCR was negative.	July 25, 2020 onward	August 19	2
31	A contact of a confirmed case. First SARS-CoV-2 RT-PCR was negative.	August 12, 2020	August 20	2
32	A contact of a confirmed case. First SARS-CoV-2 RT-PCR was negative.	July 24, 2020	August 20	2

* None presenting with any respiratory sign/symptom at the time of swab collection of mass community testing.

†1) Would have been missed without mass community testing, 2) could be picked up through contact tracing and PCR testing of contacts of a confirmed case.

We present our experience of applying a sample pooling strategy for mass community screening of SARS-CoV-2 in asymptomatic people in Da Nang city, an epicenter of the largest COVID-19 outbreak in Vietnam to date. We tested 96,123 asymptomatic individuals and found 32 confirmed cases (0.1%), accounting for 21% of 156 reported cases in Da Nang city during the same period. Of these, 22 had no history of contact with a confirmed case. Therefore, they would have been missed, had mass community testing not been implemented, and may have led to the expansion of the outbreak.

In previous reports, individual samples were separately collected, and an equal volume from up to 30 samples was then pooled into one tube before nucleic acid isolation for

subsequent RT-PCR analysis.^{7,8} This dilutes individual samples of the pools, and therefore might reduce the sensitivity of the RT-PCR,^{7,9} especially in samples with low viral loads.¹⁰ In our strategy, we combined two to seven individual swabs in one tube at the time of collection, and we observed no difference in the obtained Ct values between the pooled and individual NTS samples.

Potentially, the reduction in time required to complete the screening of 96,123 individuals from 64 days to 14 days had significant implications for the success of COVID-19 control in Da Nang. In addition, combining individual swabs at collection reduced 77% of the freezer space required to accommodate all NTS samples from those 96,123 individuals,

and substantially reduced the reagents and manpower-associated costs. These costs and time savings are critical to COVID-19 control, especially in resource-limited settings.

The success of our mass screening strategy would also depend on how soon the pools could be tested for SARS-CoV-2 by RT-PCR and for the individuals of positive pools to be resampled for retesting. In the present study, PCR analysis of pooled samples was completed within a day, allowing for the resampling and the second PCR to be carried out within the next 48 hours. Thus, we achieved a turnaround time of \leq 3 days. The turnaround time might be longer if extraction of pooled samples was unsuccessful. Although this did not happen in the present study, it would require the resampling of all individuals and is a potential limitation of the strategy.

To summarize, we showed that combining NTS samples at the time of collection allows for high throughput mass screening of asymptomatic individuals, while maintaining the sensitivity and substantially reducing the associated costs. The approach might be applicable in other settings, where there are shortages of diagnostic reagents and the disease prevalence is low, but the demand for testing is high.

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