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Original Article

Evaluating the efficiency and economic effect of two specimen pooling strategies for SARS-CoV-2 RNA detection

Wenfeng Gong[1], Yong Chen[2,3], Xiangyan HE[2,3], Xing Weng[2,3], Yang Lin[2,3], Xiaowei Xiao[2,3], Zengrong Ding[2,3], Lingling Yang[2,3], XiaoYan Ren[7], Honglong Wu[4,2]*, and Weijun Chen[2,5,6]*

[1]*Department of International Health, Johns Hopkins University, China*

[2]*BGI PathoGenesis Pharmaceutical Technology, BGI-Shenzhen, China*

[3]*BGI Wuhan Biotechnology, BGI-Shenzhen, China*

[4]*Wuhan National Laboratory for Optoelectronics, Key Laboratory of Information Storage System, Huazhong University of Science and Technology, China*

[5]*Clinical laboratory of BGI Health, BGI-Shenzhen, China*

[6]*China BGI education center, University of Chinese Academy of Sciences, China*

[7]*Clinical Laboratory of Renqiu Friendship Hospital, China*

***Corresponding author**

Jean Fabrice YALA. Laboratoire de Biologie Moléculaire et Cellulaire ; Équipe de Microbiologie, Université des Sciences et Techniques de Masuku (USTM), BP 067 Franceville, Gabon. Correspondant, Tel : +241 66.18.84.88; Email : yalajeanfabrice@gmail.com

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Abstract

The recent emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) led to the ongoing global pandemic and highlighted the need of early diagnosis of emerging infectious diseases. To control the spreading of the disease, rapid diagnosis of SARS-CoV-2 infection and large-scale population screening are required. The pooling test is an economical and effective method to improve the detection capacity of medical laboratories and reduce the demands for laboratory resources such as laboratory workers, testing reagents and equipments. This study aimed to provide an efficient method of specimen pooling by assessing the sensitivity of dry pooling and wet pooling strategies for the detection of SARS-CoV-2 virus. The dry pooling means that a certain number of throat swaps are collected together into a tube with 3 ml viral transport media and mixed, while wet pooling means that throat swap is individually collected into a tube with 3 ml viral transport media and then 200 ul media taken from each sample are mixed together in another tube. The results showed that the deviation of Ct values between dry pooling groups is smaller than that between wet pooling groups. To maintain the sensitivity of detection, for the wet pooling strategy, the number of samples to be pooled is recommended to be 5. Interestingly, 5- or 10-sample dry pooling methods both don't impact the detection sensitivity. Pooling test for detection of SARS-CoV-2 infection in regions with low Covid-19 incidence rates (≤ 1%) can dramatically decrease the cost of testing by up to 75%.

ABBREVIATIONS

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; **PPE:** personal protective equipment; **VTM:** viral transport media

INTRODUCTION

The ongoing pandemic of coronavirus disease 2019 (COVID-19) has a serious impact in the world and highlighted the need for early diagnosis of emerging infectious diseases. As of 4th March, 2021, there have been 114,653,749 confirmed cases of COVID-19, including 2,550,500 deaths, reported to WHO. (https://covid19.who.int/). Rapid diagnosis of SARS-CoV-2 is the most important method for identifying infected individuals, specifically for the asymptomatic infections [1]. Large-scale epidemiological investigation for the community population is significant to discover the asymptomatic infections and create good conditions for the resumption of work and school. So far, RT-PCR is the gold standard for the detection of SARS-CoV-2 infection and it is usually performed with individual samples. However, the individual testing mode has big drawbacks, such as limited detection capacity resulting in low screen efficiency. Therefore, faster and more effective strategies to deal with the emergency are urgently required.

To enhance the detection capacity, many researchers have proposed a " specimen pooling " detection strategy which means that multiple individual samples were collected and mixed and then tested together in a single reaction by RT-QPCR. Viral RNA was extracted and tested using the standard real-time quantitative PCR (qPCR) to assess the detection sensitivity of pooling strategies. If the pooled sample was tested positive for

SARS-CoV-2, the persons corresponding to the individual samples which were mixed to create the pooled sample will need to be tested again respectively to figure out who carries SARS-CoV-2 virus. Negative pools did not need to test again. Specimen pooling strategy will help to improve test efficiency and simultaneously reduce testing costs. Specimen pooling testing methods are more economical than individual sample testing method when they are applied in regions with low Covid-19 incidence rates. The pooling test is an approach which improves the testing capacity of clinical laboratories and reduce the reagent consumption. However pooling test may result in reduced sensitivity and decreased accuracy of SARS-CoV-2 RT-PCR tests. To assess this, the sensitivities of several different sample pooling methods were evaluated. There are different sample pooling strategies to detect SARS-CoV-2 [2-7].

In this study, throat swabs were collected from 21 asymptomatic infected persons who lived in Wuhan, China. 5- or 10-sample dry pooling and 5- or 10-sample wet pooling tests were performed for the detection of SARS-CoV-2virus.The statistical significance of the Ct value difference between the pooled sample group and the individual specimen group was analyzed. The aim of our study is to evaluate which pooling test strategy has better performance including stability as well as sensitivity and requires lower cost. Hence, the stability and sensitivity of different pooling strategies were evaluated, and besides the cost-effectiveness of each method was also analyzed based on the varying disease prevalence rates (0.1%-5%) in 1 million people.

MATERIALS AND METHODS

This study is a performance assessment of pooling test for the detection of SARS-CoV-2 infection using clinical specimens. Throat swabs from 21 asymptomatic SARS-CoV-2 infected patients who live in Wuhan were collected individually into tubes containing 3 ml viral transport media. All specimens were stored at -80°C before the SARS-CoV-2 testing which took place between 26 April and 18 May, 2020. The leftover specimens were used as individual samples (non-pooled samples, the matched controls of sample pooling groups) in this study. 14 positive samples and 64 negative samples from 13 volunteers were collected for the dry pooling test. One positive sample (swab) combined with 4 or 9 negative samples (swabs) were put into 3 ml viral transport media, and each type of pooling (5-sample or 10-sample pooling) has 7 groups. For the wet pooling test, 7 positive samples and 13 negative samples were collected and each sample was separately stored in 3 ml viral transport media. And 200 µl of VTM (viral transport media) from positive specimen was mixed with 800 µl or 1800 µl of VTM from 4 or 9 negative specimens respectively. 14 wet pooling sample groups are formed, including 7 for 5-sample pooling and 7 for 10-sample pooling. Each sample group is triplicated. The experimental design was illustrated in [Figure 1].

Viral RNA was extracted from 300 µl specimens using a separate extraction system (EZ1, Qiagen, Hilden, Germany) according to the manufacturer`s instructions. Real-time PCR (qPCR) for detection of SARS-CoV-2 was performed using a commercial kit designed to amplify the ORF1ab gene according to the manufacturer's protocol (BGI, Shenzhen, China). The limit of detection for SARS-CoV-2 gene ORF1ab was 100 copies/mL, and the cutoff of PCR cycle threshold (Ct) was 38. The ORF1ab gene fragment was amplified using probe primer 5'-FAM-TGCCACTTCTGCTGCTCTTCAACC-BHQ1-3', primer NPC1-YF11 5'-CAAGGTAAACCTTTGGAATTTG3' and NPC1-YR12: 5'-TTGTCCTCACTGCCGTCTTG-3'. Briefly, 10 µl of RNA extracted from the sample was mixed well with 18.5 µl SARS-CoV-2 reaction solution as well as 1.5 µl SARS-CoV-2 detection enzyme solution before being centrifuged for Real time PCR. Simultaneously, equivalent volumes of positive control and blank control should be processed identically. The PCR reaction parameters were as follows: cDNA synthesis by reverse transcription for 20 min at 50°C, pre-denaturation for 5 min at 95°C, followed by 45 cycles of denaturation for 15 s at 95°C, annealing for 30 s at 60°C.

The Ct valves in SARS-CoV-2 RT-PCR assays of individual and pooling samples were analyzed and compared. Statistical significance of differences among groups were detected by one-

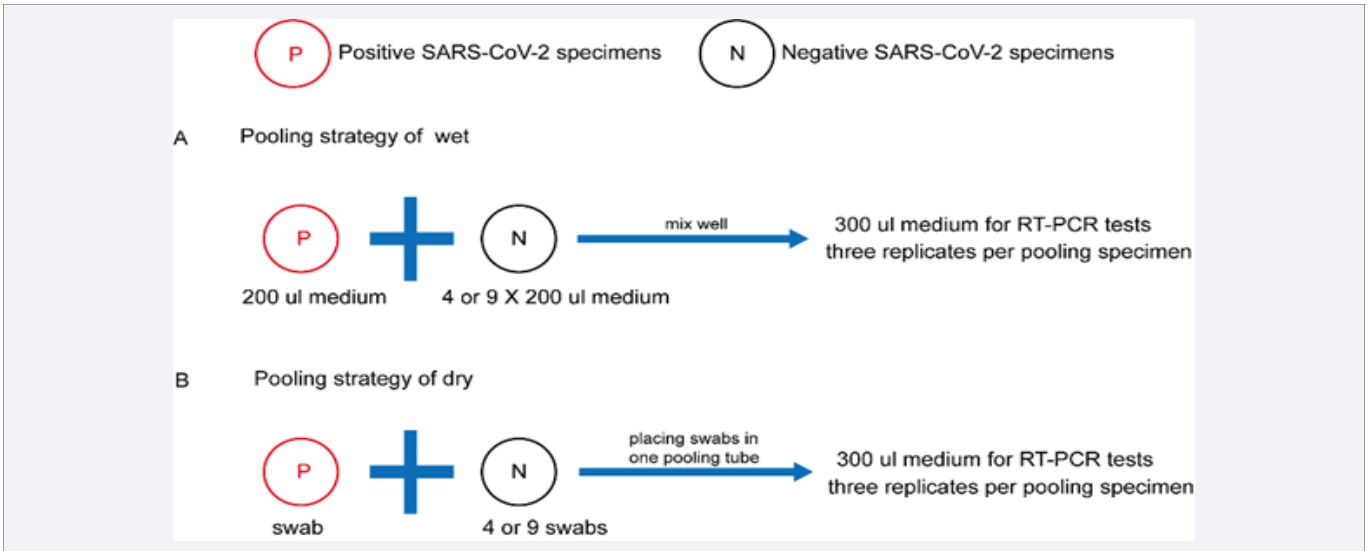

Figure 1 Schematic illustrates the experimental design of the pooling strategies. (A) The wet pooling strategy of 5 or 10 individual specimens. (B) The dry pooling strategy of 5 or 10 swabs from individual patients.

way analysis of variance. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Statistical paired t test was calculated to compare the Ct value differences between individual specimen, dry-pooling specimen, and wet-pooling specimen testing. The results showed that there was no significant difference between the three groups [Figure 2].

The differences of the CT values between 5-sample wet pooling groups (CT value >38) and individual (non-pooled) samples ranged from -0.32 to +5.02. [Table 1]. One or two replicates from 3 out of 7 wet pooling groups with size 10 has CT value less than 38, suggesting that the wet pooling size should not exceed 5 in RT-PCR assay for the detection of SARS-CoV-2. The statistical significances of the Ct value difference between the wet pooled sample group and the individual specimen group was analyzed by paired t-test. The results showed no significant difference in all comparisons between individual sample groups and 5-sample wet pooling groups (P-value from 0.089 to 0.315) except the group D with a P-value of 0.028. The CT value in all 10-sample pooling groups were significantly different from that in non-pooled sample groups (P-value <0.05).

For dry pooling groups, all 7 pooling groups with size 5 and 10 (Ct value) were tested positive, with Ct value differences within a range of -0.86 to +1.68 and -3.54 to +5.99 respectively compared to that of individual samples [Table 1]. The results revealed that there were no significant differences in the CT values between individual sample groups and dry pooling groups including 5-sample pooling and 10-sample pooling. The

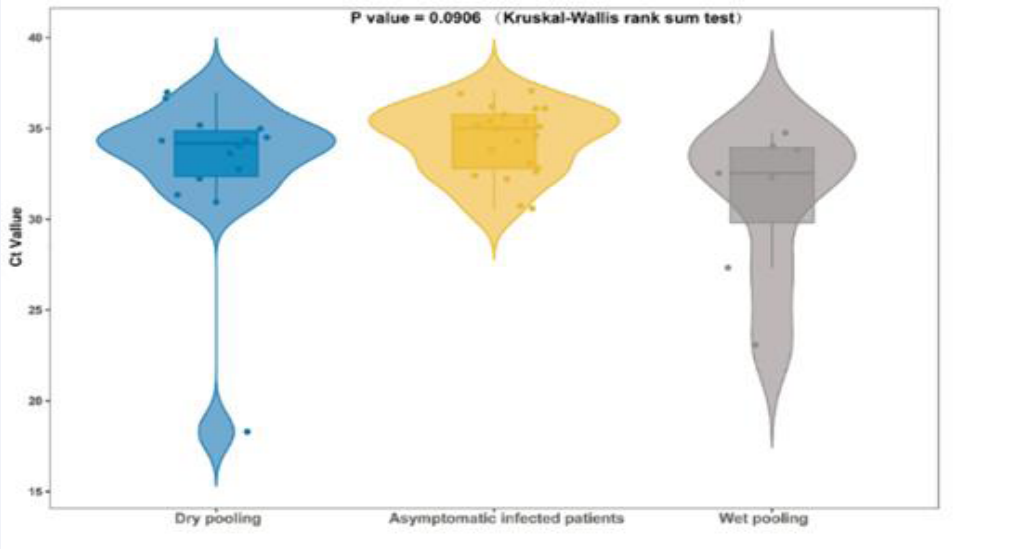

Figure 2 Comparison of Ct value of wet pooling strategy, dry pooling strategy and asymptomatic infected patients.

Table 1: Comparison of Ct value of individual with wet pooling and dry pooling strategy in pool sizes 5 and 10.

Pooling Group	Replicates	Individual Testing Ct Value		Pooled Testing Ct Value		Ct Difference (Pooled – Individual Testing)		P-value (T-test)	
		for 10 pooling	for 5 pooling	10 Samples	5 Samples	10 Samples	5 Samples	10 Samples	5 Samples
W-A	W-A1	35.04	35.04	38.22	36.81	3.18	1.77	0.021	0.157
	W-A2	34.88	34.88	37.48	35.22	2.60	0.34		
	W-A3	34.32	34.32	39.73	35.40	5.41	1.08		
W-B	W-B1	32.13	32.13	NoCt	33.92	NA	1.79	0.007	0.229
	W-B2	31.56	31.56	38.75	33.71	7.19	2.15		
	W-B3	33.91	33.91	38.14	33.59	4.23	-0.32		
W-C	W-C1	34.11	34.11	38.10	34.15	3.99	0.04	NA	0.315
	W-C2	34.31	34.31	NoCt	34.47	NA	0.16		
	W-C3	33.67	33.67	NoCt	36.82	NA	3.15		
W-D	W-D1	34.41	34.41	35.50	35.78	1.09	1.37	0.014	0.028
	W-D2	33.88	33.88	36.75	34.87	2.87	0.99		
	W-D3	33.22	33.22	35.74	35.39	2.52	2.17		
W-E	W-E1	30.26	30.26	NoCt	35.28	NA	5.02	0.037	0.131
	W-E2	33.42	33.42	37.16	34.62	3.74	1.2		
	W-E3	33.18	33.18	39.30	34.19	6.12	1.01		

W-F	W-F1	25.61	25.61	32.40	29.80	6.79	4.19	0.022	0.089
	W-F2	27.91	27.91	32.38	30.23	4.47	2.32		
	W-F3	28.46	28.46	32.98	29.99	4.52	1.53		
W-G	W-G1	21.38	21.38	28.67	24.73	7.29	3.35	0.020	0.129
	W-G2	23.74	23.74	28.19	25.97	4.45	2.23		
	W-G3	24.11	24.11	28.76	24.53	4.65	0.42		
D-A	D-A1	36.54	34.38	37.91	35.39	1.37	1.01	0.449	0.699
	D-A2	37.86	34.81	37.67	34.03	-0.19	-0.78		
	D-A3	36.53	34.3	36.77	34.62	0.24	0.32		
D-B	D-B1	37.06	32.72	36.95	33.01	-0.11	0.29	0.820	0.326
	D-B2	36.79	32.05	36.03	33.73	-0.76	1.68		
	D-B3	36.1	33.49	37.32	33.17	1.22	-0.32		
D-C	D-C1	34.61	33.53	35.76	34.74	1.15	1.21	0.168	0.561
	D-C2	35.48	33.61	35.68	33.95	0.2	0.34		
	D-C3	35.45	33.73	35.88	33.14	0.43	-0.59		
D-D	D-D1	34.03	34.05	35.56	34.54	1.53	0.49	0.921	0.395
	D-D2	36.46	33.99	34.79	34.63	-1.67	0.64		
	D-D3	34.42	34.97	34.82	34.87	0.4	-0.1		
D-E	D-E1	33.74	31.83	35.59	31.28	1.85	-0.55	0.573	0.513
	D-E2	30.54	31.19	36.53	31.05	5.99	-0.14		
	D-E3	37.82	31.04	34.28	31.15	-3.54	0.11		
D-F	D-F1	31.67	34.27	33.09	34.31	1.42	0.04	0.100	0.933
	D-F2	32.63	33.81	34.25	34.73	1.62	0.92		
	D-F3	32.35	34.87	32.82	34.01	0.47	-0.86		
D-G	D-G1	30.76	18.29	30.96	18.29	0.2	0	0.315	0.268
	D-G2	30.91	18.25	31.09	18.43	0.18	0.18		
	D-G3	31.17	18.32	31.4	18.33	0.23	0.01		

Table 2: Cost comparison of pooling strategy using for SARS-CoV-2 RNA detection in pool sizes of 5 at 3 different prevalence rates.

Total population	**1,000,000 samples**		
prevalence rates (%)	0.1	1	5
The non-infected samples (%)	99.9	99	95
Number of samples per pool	5	5	5
Total number of pooling	200,000	200,000	200,000
Pool with no infection (%)	99.50	95.10	77.38
Total number of pooling without an infection	199,002	190,198	154,756
Total number of pooling with an infection	998	9,802	45,244
Number of samples that need to be tested individually after pooled qPCR	4,990	49,010	226,219
Total number of tests that need to be Performed	204,990	249,010	426,219
Cost per test (USD, $)	20	20	20
Total cost of individual testing (USD, $)	20,000,000	20,000,000	20,000,000
Total cost of specimen pooling (USD, $)	4,099,800.20	4,980,199.00	8,524,381.25
Discount (%)	79.50	75.10	57.38
Cost per patient (USD, $)	4.10	4.98	8.52

difference (P-value) in CT values between 5-sample pooling groups and individual sample groups is less than that between 10-sample pooling groups and non-pooled sample groups. The good performance of RT-PCR for the detection of SARS-CoV-2 in dry pooling groups indicated that dry pooling strategy could be used for large-scale population screening in regions with low infection rates. However, this need more experimental data to prove.

Cost-effectiveness of pooling sample strategy was calculated, based on varying disease prevalence rates (0.1%-5%) in 1 million samples [Table 2] . It was showed that pooling sample method is acost-effective strategy to be adopted to detect SARS-CoV-2 infection in populations with lower COVID-19 prevalence. The estimated costs for laboratory test were reduced from $20 per patient to $4.10, $4.98, and $8.52 respectively at the prevalence rate of COVID-19 in the tested population of 0.1%, 1%, and 5%.

According to this estimation, pooling sample test of 1,000,000 people in a population with 1% COVID-19 prevalence rate would save approximately $15.02 million [Table 2]. Hence, pooling test for SARS-CoV-2 infection in low COVID-19 prevalence (≤ 1%) regions can dramatically decrease the resources consumption by up to 75% and increase surveillance capabilities of the Clinical laboratory.

CONCLUSION

Pooling test is an approach to effectively improving the detection capability for SARS-CoV-2. In this study, we showed that dry pooling and wet pooling (pooling size 5) could efficiently increase surveillance coverage and capacity and do not compromise the sensitivity for detecting SARS-CoV-2. For example, in the epidemic at prevalence of 1%, pooling testing for the detection of SARS-CoV-2 infection can dramatically reduce the cost of laboratory operation by about 75% [Table 2], and in the epidemic at prevalence of 5%, pooling testing will save 57.38% cost. Compared to wet pooling method, dry pooling method can reduce viral transport media reagents which decreases the cost. Pooling test can efficiently increase test throughput, especially, in the regions with massive testing requirements. The limitation of pooling sample method is that its application is confined to the detection of SARS-CoV-2 in regions with low COVID-19 prevalence rates. A shortage of this study is that the positive specimens used to create pooled sample groups have a narrow distribution of Ct values.

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REFERENCES

1. Cohen J, Kupferschmidt K. Countries test tactics in 'war' against COVID-19. Science. 2020; 367: 1287-1288.
2. Abdalhamid B, Bilder CR, McCutchen EL, Hinrichs SH, Koepsell SA, et al. Assessment of Specimen Pooling to Conserve SARS CoV-2 Testing Resources. Am J Clin Pathol. 2020; 153: 715-718.
3. Ben-Ami R, Klochendler A, Seidel M, Sido T, Gurel-Gurevich O, Yassour M, et al. Large-scale implementation of pooled RNA extraction and RT-PCR for SARS-CoV-2 detection. Clin Microbiol Infect. 2020; 26: 1248-1253.
4. Chen F, Geng Z, Wang J, Liuchang W, Huang D, Xu Y, et al. Comparing two sample pooling strategies for SARS-CoV-2 RNA detection for efficient screening of COVID-19. J Med Virol. 2021; 93: 2805-2809.
5. Hogan CA, Sahoo MK, Pinsky BA. Sample Pooling as a Strategy to Detect Community Transmission of SARS-CoV-2. JAMA. 2020; 323: 1967-1969.
6. Praharaj I, Jain A, Singh M, Balakrishnan A, Dhodapkar R, Borkakoty B, et al. Pooled testing for COVID-19 diagnosis by real-time RT-PCR: A multi-site comparative evaluation of 5- & 10-sample pooling. Indian J Med Res. 2020; 152: 88-94.
7. Wacharapluesadee S, Kaewpom T, Ampoot W, Ghai S, Khamhang W, Worachotsueptrakun K, et al. Evaluating the efficiency of specimen pooling for PCR-based detection of COVID-19. J Med Virol. 2020; 92: 2193-2199.

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