Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Clinica Chimica Acta 521 (2021) 155–157

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Letter to the editor

A theoretical simulation of SARS-CoV-2 pooled testing: Pooled sample collection outperforms pooled RNA extraction

ARTICLE INFO

Keywords
Pooled testing
Pooled sample collection
SARS-CoV-2
COVID-19

To the Editor,

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2), has resulted in more than 175 million confirmed cases, including 3.8 million deaths since its first detection in December 2019 [1]. Unfortunately, there are still a great many new cases identified every day worldwide. As in the Boulder cohort, just 2% of SARS-CoV-2 asymptomatic infected individuals harbored 90% of the virus circulating in communities and served as possibly viral super-carriers and super-spreaders [2]. Therefore, identifying these infected individuals is urgently imperative to prevent a growing global outbreak. Widespread diagnostic testing is an efficient way to control virus spread, and it has promoted the emergence of high throughput pooled testing in the context of the COVID-19 pandemic [2–8]. Pooled testing has proven as an effective strategy for large-scale SARS-CoV-2 screening [4,5]. Mina et al. utilized an optimized combinatorial pooling testing strategy for population screening, which estimated the prevalence across a broad range from 0.02% to 20% [4]. A COVID-19 diagnosis team of Hebrew University Hadassah tested 133,816 clinical samples using the Dorfman pooling testing for the presence of SARS-CoV-2 and spared 76% of the RNA extraction and PCR tests compared with individual testing, with an acceptable reduction in sensitivity [5]. Ndifon et al. employed a hypercube algorithm to design a pooling scheme and achieved a large group size of up to 100 samples [6]. These researches strongly support the use of pooling tests for large-scale COVID-19 screening.

However, one of the weak points of these reported pooling tests is that they bring sensitivity losses. Herein, a novel pooled testing strategy for SARS-CoV-2 screening was proposed by pooling several individual swabs in a sampling tube before performing RNA extraction and detection. In a pooled testing with a pool size of 5, this testing strategy shows a greater than 3-fold increase in test sensitivity and saves 80% reagents compared with the conventional Dorfman pooling strategy.

We simulated and characterized the pooled testing by dipping oropharyngeal swabs into SARS-CoV-2 pseudovirus solution, moving the swab to viral transport medium (VTM), and sampling 200 μL of the VTM to perform the RNA extraction and RT-qPCR test. The sample pooling strategies used in this study are shown in Fig. 1A–C. The detailed methods for simulating pooled testing of SARS-CoV-2 are demonstrated in supplementary material.

Conventional pooled testing, in which each sample is collected individually, followed by pooling several samples before RNA extraction and RT-qPCR detection, always brings a sensitivity loss due to the dilution of pooling samples (Fig. 1A) [9,10]. For the screening testing of the COVID-19 carriers, a highly sensitive pooled testing strategy is necessary, particularly for these individuals with lower viral load. Accordingly, a novel pooled testing scheme was developed to reduce the sensitivity loss of the pooled testing. As shown in Fig. 1B, sample pooling is performed at the time of sample collection by assembling several swabs in a sampling tube, followed by performing RNA extraction and detection. In this strategy, if a sampling tube includes more than two positive samples, the virus particles will be concentrated instead of being diluted compared with the conventional pooling strategy. Additionally, this strategy enables the release of substantial laboratory manipulations and spares a large number of reagents (Table S1). Only if a pooled sampling tube tests positive, each sample in this tube needs to be retested individually. There are two alternatives for sample retest in strategy B. (1) Re-collect specimen individually and then perform a single retest; (2) Collect two swab specimens at one time, one is stored in the pooled sampling tube for pooled testing, and the other one is stored in another single tube for the possible retest. To further expand the test capacity, we devised another pooling strategy: pooled sample collection followed by pooled RNA extraction. As depicted in Fig. 1C, multiple samples are firstly collected in a sampling tube, and then they are pooled to perform RNA extraction and detection.

We evaluated the sensitivity losses of these three pooling schemes by simulating the sample collection via the use of oropharyngeal swab and SARS-CoV-2 pseudovirus. Sensitivity loss was firstly evaluated in strategies A and B with pool sizes of 3, 5, 8, and 10, respectively. Comparing with the single test, the sensitivities of the pooled testing in strategies A and B both decreased (Fig. 1D and E). The Ct values of samples in strategy A exponentially increased with the pool size increasing, which was resulted from the dilution of pooling multiple samples. While for strategy B, the increased Ct values for samples with various pool sizes were almost the same, showing a mean value of 2.78 and 1.67 for

https://doi.org/10.1016/j.cca.2021.07.016
Received 1 July 2021; Received in revised form 13 July 2021; Accepted 13 July 2021
Available online 16 July 2021

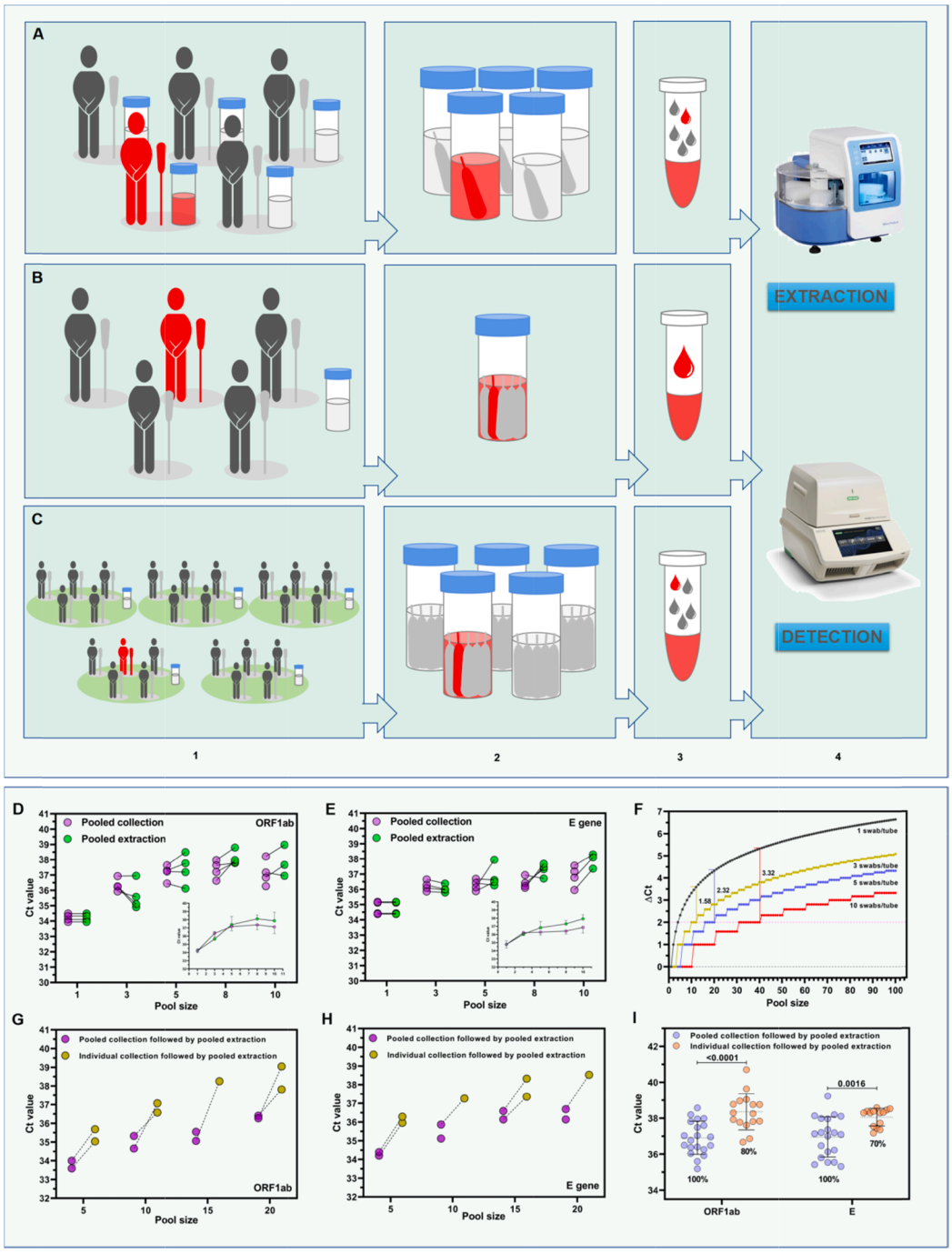

Fig. 1. The diagram of three various pooled testing strategies (A, B and C); four stages are included in a routine workflow for SARS-CoV-2 test, stages 1 to 2 indicate sample collection, stage 3 indicates sample pooling, and stage 4 indicates RNA extraction and detection. Comparison of the sensitivity loss between pooling strategy A and B using 10^4 copies/mL of the SARS-CoV-2 pseudovirus (D and E). The increased Ct ($\Delta Ct = Ct_{pool\ test} - Ct_{single\ test}$, one pool contains one positive sample) is a function of pool size, 3 swabs/tube indicates that a sampling tube contains ≤3 oropharyngeal swabs, while 5 swabs/tube and 10 swabs/tube represent that the number of swabs in a sampling tube are ≤5 and ≤10, respectively (F). Comparison of the sensitivity loss between pooling strategy A and C (G and H). Assessment of the test reproducibility by using a *t* test, showing that the identified positive samples in pooling strategy A is statistically significant in comparison with that in strategy B using 20 replicates (I).

amplifying the ORF1ab and E genes of SARS-CoV-2, which were lower than that in strategy. Theoretically, the Ct values of samples with various pool sizes in strategy B should not increase, but actually, an increase was observed in our study. We inferred that this increase possibly resulted from the adsorption of negative swabs to virus particles. However, strategy A shows a significant decrease in sensitivity compared with strategy B, and sensitivity losses became greater and greater with the sample pool size increasing.

Next, we calculated the change of Ct in strategy C with the sample pool size increasing from 2 to 100 under various pooled sample collection sizes. As shown in Fig. 1F, if 12 samples were pooled, we observed that the ΔCt was 3.59 for samples collected individually; while that for samples with collection pool sizes of 3, 5, and 10 were 2.00, 1.59, and 1.00, respectively. With 40 samples in a pool, the ΔCt we found in strategy A was 5.32, and that for samples in strategy C with collection pool sizes of 3, 5, and 10 were 3.81, 3.00, and 2.00, respectively. These data strongly suggest that the loss of sensitivity in strategy C was smaller than that in strategy A. Moreover, the pooled testing strategy C with a pooled collection size of 5 was validated in the SARS-CoV-2 pseudovirus sample. As shown in Fig. 1G and H, the Ct values of the samples tested using strategy C were lower than those using strategy A. We also observed that the test results of the pooled testing using strategy A were inconclusive when the pool size was greater than 10, while that for strategy C was robustly stable. In comparison with using strategy A for pooled testing, we found that the decreased Ct were about 2.10 and 2.11 for amplifying the ORF1ab and E genes using strategy C with a final pool size of 10, which was very close to the theoretical value of 2.32 (Fig. 1F–H). Last but not least, in a 10 -sample pool, the test

reproducibility of the strategies A and C with a sample collection size of 5 was evaluated with 20 replicates. The result shows that the Positive Predictive Value (PPV) was 100% for amplifying the ORF1ab and E genes in strategy C, while that for strategy A was 80% and 70%, respectively (Fig. 1I). In addition, we also observed a significant decrease of the Ct in strategy C compared with that in strategy A, showing a decreased Ct of 1.45 ± 0.32 cycles and 1.10 ± 0.32 cycles for amplifying ORF1ab and E genes, respectively. These results suggest that the loss of sensitivity in strategy C was far less than that in strategy A, particularly for the large pool sizes.

In brief, we preliminarily evaluated the performances of three pooled testing strategies in this study, both strategies A and B have been put into practice in many countries. Strategy C is a promising alternative for large-scale COVID-19 screening testing in resource-limited settings.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was financially supported by grants from China MoST Emergency Project on COVID-19 (2020YFC084800).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2021.07.016.

References

[1] World Health Organization, WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int.

[2] Q. Yang, T.K. Saldi, P.K. Gonzales, et al., Just 2% of SARS-CoV-2-positive individuals carry 90% of the virus circulating in communities, e2104547118, Proc. Natl. Acad. Sci. U. S. A. 118 (2021), https://www.pnas.org/content/118/21/e2104547118.

[3] C. Brian, A.H. James, B. Brendan, et al., Using viral load and epidemic dynamics to optimize pooled testing in resource-constrained settings, Sci. Transl. Med. 13 (2021) eabf1568. https://stm.sciencemag.org/content/13/589/eabf1568.

[4] B. Netta, B.A. Roni, S. Tal, et al., Lessons from applied large-scale pooling of 133816 SARSCoV-2 RT-PCR tests, Sci. Transl. Med. 13 (2021) eabf2823. https://stm.sciencemag.org/content/13/589/eabf2823.

[5] M. Leon, N. Pacifique, B. Yvan, et al., A pooled testing strategy for identifying SARS-CoV-2 at low prevalence, Nature 589 (2021) 276–280. https://www.nature.com/articles/s41586-020-2885-5.

[6] S. Noam, L. Shlomia, W. Vered, et al., Efficient high-throughput SARS-CoV-2 testing to detect asymptomatic carriers, Sci. Adv. 6 (2020) eabc5961. https://advances.sciencemag.org/content/6/37/eabc5961.

[7] L. Stefan, P. Thorsten, B.G. Barbara, et al., Pooling of samples for testing for SARS-CoV-2 in asymptomatic people, Lancet Infect. Dis. 20 (11) (2020) 1231–1232. https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30362-5/fulltext.

[8] J. Alcoba-Florez, H. Gil-Campesino, D. García-Martínez de Artola, O. Díez-Gil, A. Valenzuela-Fernández, R. González-Montelongo, L. Ciuffreda, C. Flores, Increasing SARS-CoV-2 RT-qPCR testing capacity by sample pooling, Int. J. Infect. Dis. 103 (2021) 19–22. https://www.ijidonline.com/article/S1201-9712(20)32468-1/fulltext.

[9] Y. Idan, A. Noga, S.T. Einat, et al., Evaluation of COVID-19 RT-qPCR test in multi sample pools, Clin. Infect. Dis. 71 (2020) 2073–2078. https://academic.oup.com/cid/article/71/16/2073/5828059.

[10] R. Ben-Ami, A. Klochendler, M. Seidel, et al., Large-scale implementation of pooled RNA extraction and RT-PCR for SARS-CoV-2 detection, Clin. Microbiol. Infect. 26 (2020) 1248–1253. https://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(20)30349-9/fulltext.

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