A low-cost and high-efficiency 10-in-1 test for the PCR-based screening of SARS-CoV-2 infection in low-risk areas

Xiaosong Qin¹, Peng Gao², Zhijie Zhang¹, Zhijian Bo³, Shijun Li⁴, Mei Yang¹, Jianhua Liu¹, Lina Wu¹, Ting Li¹, Zuowei Zhao⁴, Yan Li¹,⁶, Haodong Bian¹, Yong Liu¹

¹Department of Laboratory Medicine, Shengjing Hospital of China Medical University, Shenyang, Liaoning 110004, China;
²Department of Laboratory Medicine, Dalian Sixth People’s Hospital, Dalian, Liaoning 116031, China;
³Microbiological Laboratory, Dalian Municipal Center for Disease Control and Prevention, Dalian, Liaoning 116019, China;
⁴Department of Laboratory Medicine, First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning 116011, China;
⁵Committee of the Party Group, Health Commission of Dalian, Dalian, Liaoning 116006, China;
⁶Clinical Laboratory and Medical Quality Control Center, Health Service Center of Liaoning Province, Shenyang, Liaoning 110005, China.

To the Editor: As of August 9, 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected 19,432,244 people and caused 721,594 deaths globally. To timely identify the infected persons, nucleic acid detection screenings have been carried out for high-infection risk groups in areas known to have coronavirus disease 19 (COVID-19) cases, such as Wuhan, Beijing, and Xinjiang. The rapid identification of infected patients and the implementation of quarantine measures play an important role in preventing the transmission of SARS-CoV-2. However, there are some issues associated with large-scale screening for this virus. The consumption of equipment and reagents for specimen collection and detection is high, resulting in elevated screening costs. Moreover, with the increase in the number of samples collected, the testing capabilities of medical institutions have reached a saturation point, hindering the speed and scope of large-scale screenings. For this reason, the mixed detection after RNA extraction (detection in a mixture of nucleic acids extracted from several patients) or before RNA extraction (detection in a mixture of pharyngeal swab transfer buffer obtained from different patients before nucleic acid extraction) has been proposed in many regions.¹ However, both the pooling of swab transfer buffer before nucleic acid extraction and pooling of RNA after nucleic acid extraction inevitably cause dilution of samples and decrease of detection sensitivity. However, there is still a lack of research on how to effectively improve the screening efficiency and control the decrease of sensitivity caused by dilution in an appropriate range. In this study, we designed a novel 10-in-1 test (ten pharyngeal swab samples from ten individuals were placed in one custom-made virus collection tube [CMT] for nucleic acid extraction and testing) by optimizing the current mixed acquisition technique, and after a promising pilot test.

The study protocol was approved by the institutional review board of the Shengjing Hospital of China Medical University and Dalian Sixth People’s Hospital. We used data from a nucleic acid screening for the detection of SARS-CoV-2 launched by the local government of Dalian, which enrolled 7 million people. A total of 2.15 million subjects were included in the study; 82,000 people were used for evaluating the sampling tube usage experience (70,000 cases from 23 laboratories and 12,000 cases from 21 sample collection sites), and 640 people were used for evaluating the consistency of the methods. Among the 640 people, 64 clinical cases were confirmed COVID-19 cases from Dalian Sixth People’s Hospital (government-designated COVID-19 treatment hospital) according to the “Diagnosis and Treatment plan of Corona Virus Disease 2019.”² and 576 were volunteers who tested negative for COVID-19.

For the 10-in-1 test, a CMT was used. The CMT had an outer diameter and a height of (14.8 ± 0.2) mm × (100.5 ± 0.4) mm and contained 6 mL of preservation solution containing guanidinium salt. The commercially available virus CMT used for the individual acquisition tube (IAT) contained 3 mL of the same preservation solution as that used for the CMT that was deployed in the present study. The pharyngeal swab for the 10-in-1 test was a polypropylene flocking swab, with a head length of 2 cm, a diameter of 3 to 5 mm, and a breaking point distance from the head of the swab of 3 cm. The convenience of using CMT was assessed through a cell-phone applet that distributed a questionnaire to a subset of the medical staff involved...
in the screening. We received a total of 118 questionnaires from 23 laboratories and 21 sample collection sites. More than 94% of the questionnaires indicated that CMT was convenience of sampling and breaking for swab.

The pharyngeal swabs of 19 SARS-CoV-2-positive patients and asymptomatic volunteers were collected using IAT on the first day after admission to the hospital. Pharyngeal swabs were also collected from another 171 SARS-CoV-2-negative volunteers and stored in a CMT (nine swabs for each tube). Two-hundred microliters of preservation solution from each IAT and each CMT was collected and mixed well. The presence of the SARS-CoV-2 viral RNA was then extracted from the mixture and detected using real-time reverse transcription-polymerase chain reaction (RT-PCR) amplification of the viral open reading frame 1ab (ORF1ab) and nucleocapsid protein (NP) genes fragments using kits provided by Guangzhou Da’an Gene Biotechnology Co., Ltd. (Guangzhou, China, detection limit: 500 copies/mL) and Wuhan Mingde Biotechnology Co., Ltd. (Wuhan, China, detection limit: 500 copies/mL). RNA from all IAT and CMT samples was extracted within 24 h of collection using a magnetic bead-based purification method. And the cycle threshold (Ct) value of ORF1ab and NP genes was recorded. We simulated a 10-in-1 test by taking equal volumes of solutions from an IAT containing a positive swab and a CMT containing nine negative swabs and mixing them.

For the 19 COVID-19-positive samples, the Ct values for ORF1ab and NP gene amplicons of the samples collected in IAT were 28.89 ± 5.21 and 29.02 ± 5.74, respectively. Conversely, the Ct values of ORF1ab and NP gene amplicons in these samples, when mixed with nine negative samples, were 30.00 ± 5.07 and 30.03 ± 5.56, respectively. According to the RT-PCR kit instructions, the IAT samples were positive in all 19 cases, while in the 10-in-1 simulation, 18 samples were positive, and the result for the remaining cases was uncertain, as it was positive only for ORF1ab. Thus, the IAT and the simulated 10-in-1 test showed a high degree of consistency, with a positive coincidence rate of 94.7%.

Although the 10-in-1 simulation was promising, we considered that the presence of potential confounding factors, such as multiple human genes in a real 10-in-1 test solution, might negatively affect the situation on the field. Thus, we collected samples to perform a real 10-in-1 test. Two pharyngeal swabs (per person) were collected from 405 volunteers who were enrolled in the study. One swab was placed in an IAT and the other swab was put into a CMT, which contained nine different pharyngeal swabs. Two pharyngeal swabs were collected from forty-five COVID-19 positive patients. One swab was put into an IAT, and the other swab was added to the CMT already containing swabs with samples from nine healthy volunteers. Ct values of IAT and CMT samples in the real 10-in-1 test detected by different reagents (Da’an and Mingde) are shown in Figure 1. The results obtained using CMT and IAT collections were consistent, and the Kappa values from Da’an and Mingde kits were 0.863 (95% confidence interval [CI]: 0.7552, 0.9708) and 0.973 (95% CI: 1.02592, 0.92008), respectively. Moreover, the Kappa value for 450 IAT samples tested using the Da’an and Mingde kits was 0.856.
Then, we screened 2.15 million people in Dalian, 11 million in Qingdao, and 7.7 million in Shenyang using the 10-in-1 test in low-risk areas. We identified no positive cases in low-risk areas in Dalian and Qingdao, and no new patients were diagnosed with COVID-19 in the screened population since the completion of screening. While in the epidemic of Shenyang in December 2020, a case of SARS-CoV-2-positive was screened out through 10-in-1 test, and was finally confirmed to be an asymptomatic patient, all these data suggested that the 10-in-1 test-based screening was accurate and applicable.

In designing the 10-in-1 test, we encountered three problems. The first problem was to design a single CMT having the capacity to accommodate ten pharyngeal swabs without causing inconvenience to the collector. To address this problem, the outer diameter of CMT, the outer diameter and height of CMT cap, the material and length of swab rod, the material and length of swab head, and the fiber degree of swab were designed to enable the break of pharyngeal swab in a suitable position. The resulting CMT could hold ten pharyngeal swabs and meets the requirements for safe sampling and convenience of use.

The second was to increase the volume of virus preservation fluid for adequate elution of up to ten pharyngeal swabs while ensuring that the samples are not over-diluted, to not compromise the assay’s accuracy. Several studies have reported that different nucleic acid pooling strategies can be adopted in areas with different disease prevalence. They found that in low-risk areas, the sample dilution can be up to 20 or more. The CMT used in this study doubled the amount of preservation fluid used in IAT, causing a two-fold dilution. For PCR, a two-fold dilution increases the Ct value by only one. Thus, this dilution can achieve the same detection efficiency as the original, preserving the assay reliability. We first performed a simulated test using the same sample concentration as that to be used for a real 10-in-1 collection. We found a positive coincidence rate with IAT of 94.7%; however, upon re-testing, the 19th case also tested positive. At the simulated stage, the average Ct value of 19 samples obtained from patients at the early onset of disease and asymptomatic patients was around 28, indicating that the pharyngeal swab viral load in the early stage was relatively high, consistently with a previous report. These results suggested that the 10-in-1 test was feasible for COVID-19 screening.

The third was that we did not have enough new cases for a real 10-in-1 test at the beginning of study, and we used cases that had been treated for some time. This was not ideal, since the 10-in-1 test was designed to screen new cases or asymptomatic patients. Patients enrolled after treatment had improved conditions and lower viral load. Moreover, some of them had no detectable SARS-CoV-2 nucleic acids. However, according to our and other studies,[4,5] the viral load of newly infected patients with mild symptoms or being asymptomatic is usually high. Considering that even when testing samples obtained from treated patients with a low viral load, we obtained a good diagnostic agreement with IAT samples, we concluded that the 10-in-1 test should be even more reliable for screening primary cases.

Our method enables the screening of a large population with high efficiency and low cost. In the future, we aim to optimize the pooling technique and investigate the suitability of pooling more samples for large-scale population screening.

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Conflicts of interest

None.

References

1. Abdalhamid B, Bilder CR, McCutchen EL, Hinrichs SH, Koepsell SA, Iwen PC. Assessment of specimen pooling to conserve SARS CoV-2 testing resources. Am J Clin Pathol 2020;153:715–718. doi: 10.1093/ajcp/aqaa064.
2. China National Health Commission. Novel Coronavirus Pneumonia Diagnosis and Treatment Program (Trial Seventh Edition). Available from: https://www.nhc.gov.cn/yyypjg/s7653p/202003/46c9294a7dfe4cf80dc75912e8b1989.shtml. Accessed March 4, 2020.
3. Pilcher CD, Westreich D, Hudgens MG. Group testing for Sars-Cov-2 to enable rapid scale-up of testing and real-time surveillance of incidence. J Infect Dis 2020;222:903–909. doi: 10.1093/infdis/jiaa378.
4. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Mueller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020;581:465–469. doi: 10.1038/s41586-020-2196-x.
5. Liu Y, Yan LM, Wan L, Xiang TX, Le A, Liu JM, et al. Viral dynamics in mild and severe cases of COVID-19. Lancet Infect Dis 2020;20:656–657. doi: 10.1016/S1473-3099(20)30232-2.

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