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Evaluation of the clinical performance of single-, dual-, and triple-target SARS-CoV-2 RT-qPCR methods

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ABSTRACT

Background: The coronavirus disease 2019 (COVID-19) has become a pandemic. Reverse transcription quantitative PCR (RT-qPCR) has played a vital role in the diagnosis of COVID-19, but the rates of false negatives is not ideal in dealing with this highly infectious virus. It is thus necessary to systematically evaluate the clinical performance of the single-, dual-, triple-target detection kits to guide the clinical diagnosis of this disease.

Methods: A series of reference materials calibrated by droplet digital PCR (ddPCR) and 57 clinical samples were used to evaluate the clinical performance of six single-, dual-, triple-target SARS-CoV-2 nucleic acid detection kits based on RT-qPCR.

Results: The dual-target kits, kit B and kit C had the highest and the lowest detection sensitivity, which was 125 copies/mL and 4000 copies/mL, respectively. Among the 57 clinical samples from patients with COVID-19, 47 were tested positive by the kit B, while 35, 29, 28, 30, and 29 were found positive by the kits A, C, D, E, and F, respectively. The number of targets in a detection kit is not a key factor affecting sensitivity, while the amount of sample loading may influence the performance of a detection kit.

Conclusions: This study provides a guide when choosing or developing a nucleic acid detection kit for the diagnosis of COVID-19. Also, the absolute-quantification feature and high-sensitivity performance of ddPCR, suggesting that it can be used to review clinically suspected samples.

1. Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), being first reported in December 2019, has rapidly infected many around the world [1]. As of MAY 13, 2020, 4 million cases of coronavirus disease 2019 (COVID-19) and two hundred thousand deaths

ABBREVIATIONS: COVID-19, Coronavirus Disease 2019; RT-qPCR, Reverse transcription quantitative PCR; ddPCR, droplet digital PCR; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; NMPA, China National Medical Products Administration

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had been reported [2]. SARS-CoV-2 is a β-type coronavirus and closely related to the bat-derived severe acute respiratory syndrome (SARS)-like coronavirus bat-SL-CoVZC45 [3]. SARS-CoV-2 is highly infectious and can be transmitted by respiratory droplets, contact, aerosols, and fecal matter [4]. It poses huge challenges for public health due to the lack of a vaccine and treatment options. Therefore, effective diagnostic methods are urgently needed.

Since the outbreak of COVID-19, China has accumulated valuable clinical experiences. One of the diagnostic criteria of “confirmed case” in the Diagnosis and Treatment Protocol for COVID-19 (trial version 7) is that the reverse transcription quantitative PCR (RT-qPCR) detection of SARS-CoV-2 nucleic acid indicates positive [5]. Additionally, the discharge criteria stated in the guidelines require two consecutive negative tests for the viral nucleic acid in respiratory samples. Therefore, nucleic acid detection is an important method to confirm the diagnosis of COVID-19 and to determine patient discharge. Currently, RT-qPCR is the most commonly used method to detect viral nucleic acids, with the advantages of being fast, easy to perform and cost-effective [6].

However, the results of the SARS-CoV-2 nucleic acid detection kits based on RT-qPCR in some patients with confirmed COVID-19 were found to be inconsistent with imaging results, suggesting that nucleic acid detection may produce false-negative results [7,8]. The detection kits detect viral RNA via targeting the conserved regions of SARS-CoV-2, such as ORF1ab, N, and E genes [9]. Some of the kits target a single gene while the others target two or three genes. The number of targets in a detection kit may affect the sensitivity, which in turn leads to false-negative results. Therefore, it is necessary to investigate the performance of single-, dual- and triple-target detection kits and to compare their diagnostic sensitivity and specificity.

Droplet digital polymerase chain reaction (ddPCR) achieves absolute quantitation and is highly sensitive [10]. In ddPCR, template DNA molecules are distributed across numerous droplets, so that the number of template DNA molecules can be quantified from the proportion of positive reactions using Poisson distribution. It can detect target molecules in a complex background with high specificity and sensitivity, making it suitable for the detection of pathogens in complex clinical samples [11].

In this paper, a series of low-concentration positive reference RNAs were established using ddPCR to thoroughly evaluate the clinical performance of single-, dual- and triple-target SARS-CoV-2 detection kits. We also evaluated these kits using 57 clinical samples that had been quantified by ddPCR.

2. Materials and methods

2.1. Participants

Throat swabs or deep sputum samples were collected from COVID-19 inpatients at the Fifth People’s Hospital of Ganzhou from January 20 to March 1, 2020. In 8 of a total 47, throat swabs or deep sputum samples were collected multiple times after the onset of symptoms. Samples were collected only once from the other patients.

2.2. Sample preparation and RNA extraction

Samples were pre-processed before RNA extraction. Throat swabs were soaked in 1 mL normal saline and then vortexed. Sputum samples were mixed with an equal volume of 4% NaOH for liquefaction. Then, 200 μL preprocessed samples were used for RNA extraction. Viral RNA was extracted using the TIANLONG NP968-C Nucleic Acid Extraction System and Viral Ex-DNA/RNA Kit according to the manufacturer’s instructions. RNA was eluted with 50 μL elution buffer and stored at −80 °C.

2.3. Reverse transcription quantitative PCR (RT-qPCR)

Different China National Medical Products Administration (NMPA)-approved SARS-CoV-2 nucleic acid detection kits (RT-qPCR) were used according to the corresponding manufacturer’s instructions. All kits perform the assay in one tube. Information about the kits is listed in Table 1.

2.4. Droplet digital PCR (ddPCR)

The ddPCR analysis was performed using the SARS-CoV-2 Nucleic Acid Detection Kit (digital PCR) (TargetingOne Corporation, Beijing, China) (Table 1). The ddPCR platform system utilized for this evaluation was the TargetingOne® Digital PCR System (TargetingOne® Corporation). The reaction system included 9 μL of PCR reagent A, 3 μL of PCR reagent B, 3 μL of the primer/probe mix, and 15 μL of RNA. Then, the 30 μL digital PCR reaction mixture and 180 μL of droplet generation oil were added into a droplet generation chip, and droplet generation was performed using a Drop Maker (TargetingOne® Corporation) following the manufacturer’s instructions. Approximately 100 μL of the resulting droplet emulsion were automatically transferred into a PCR tube and amplified on a PTC-200 Thermal Cycler (Bio-Rad, CA). The PCR amplification was performed following the manufacturer’s instructions. After PCR, the PCR tube containing the droplets was connected to a droplet detection chip, hence the Chip Reader (TargetingOne® Corporation) may detect the fluorescent signals of droplets. Finally, the data were subjected to Poisson distribution analysis using the TargetingOne® 2019-nCoV software to obtain the viral copy number in the samples.

The RT-qPCR and ddPCR detection of the clinical samples were performed simultaneously. The technicians did not know whether the clinical samples were collected from patients with or without COVID-19.

2.5. Preparation of reference positive control RNA

Samples with a viral load of > 1000 copies/μL as detected by the ddPCR were used as reference substance materials. Two batches of 9
serial 2-fold dilutions of reference positive control RNA were prepared, from 32 to 0.125 copies/μL. After quantification by ddPCR to ensure the dilutions and linearity, these samples were used as reference positive control RNAs for RT-qPCR detection kit evaluation. To ensure the quality of the RNA, only RNAs stored at 4 °C for <12 h or those stored at −80 °C and subject to <3 times of freezing and thawing were used in RT-qPCR.

3. Results

3.1. Evaluation of the performance of the single-, dual-, and triple-target RT-qPCR kits using positive control RNA

As shown in Table 1, kit A is single-target detection kit; kits B–E are dual-target detection kits; kit F is triple-target detection kit. To test the sensitivity of these RT-qPCR kits, we used two serial dilutions of positive control RNAs (0219 and 0222; Table 2). The sensitivity of the kits using the 0219 positive control RNA was as follows: kits A, B, and D, 250 copies/μL; kits C and F, 500 copies/μL; and kit E, 1000 copies/μL. To further investigate the sensitivity and stability of the kits, the 0222 positive control RNA, which contained a lower concentration of viral RNA (125 copies/μL) than 0219, was used. Two replicates were performed for each dilution. As shown in Table 2, the kit B has the highest detection sensitivity, which was 125 copies/μL. However, only the N gene was detected in both replicates. The sensitivities of the kits A, C, D, E, and F were 500, 4000, 500, 1000, and 250 copies/μL, respectively. Except for kit B, the other RT-qPCR kits produced inconsistent results for the two replicates at low concentrations. In addition, all RT-qPCR kits except the kit E had different limits of detection when using two positive control RNAs.

Next, we plotted the relationship between viral RNA concentration and Ct value to analyze the stability of the RT-qPCR kits (Fig. 1). As shown in Fig. 1, with decreasing concentration, the Ct value increased linearly for kit B. The Ct values of the kits A and D showed abnormal results (showed in dot circles in Fig. 1; e.g. ORF1ab-1 of kit A and N-2 of kit D), where the Ct value of a low concentration RNA was lower than that of a high concentration RNA. Kit C only detected the N and ORF1ab genes at high concentrations. Moreover, the Ct value of the kit E fluctuated at low concentrations. The ORF1ab-2 had a lower Ct value than the other ORF1ab genes for the kit F.

As one of the dual-target detection kits (kit B) had the highest sensitivity and the best stability and other dual-target detection kits had different performance, the performance of the kit was not directly related to the number of targets. Furthermore, these results indicated that the detection of samples with a low concentration of RNA (approaching the limit of detection) was unreliable.

3.2. Demographic and clinical characteristics of the total cohort

A total of 57 samples were collected from 47 patients with COVID-19 (Table 3). For six of these patients, two samples were collected per patient on different days after the onset of symptoms; for another two patients, three samples were collected per patient on different days after the onset of symptoms. The median age of the study population was 47 years (16–80 years) and 22 patients were female. Six patients had no fever or cough, four patients had fever only, and the remaining 37 had fever and cough. In addition, two patients had high blood pressure and the others had no severe chronic disease. Sampling time was from day 1 to day 24 after the onset of symptoms and types of samples were induced sputum (n = 49) and throat swabs (n = 8). Using ddPCR, we further classified the samples as “strongly positive,” “weakly positive 1,” “weakly positive 2,” and “negative” according to their viral load (Table 4). As shown in Table 4, four samples were strongly positive (>1000 copies/15 μL); 34 samples were weakly positive (5–303 copies/15 μL); 19 samples were negative (<5 copies/15 μL).

3.3. Test of the clinical samples using the single-, dual-, and triple-target RT-qPCR kits

To further evaluate the clinical performance of the RT-qPCR kits, we used them to test all 57 samples; the results are shown in Table 5. The single-target RT-qPCR kit, kit A identified 35 samples as positive, the dual-target kits, kits B, C, D, and E identified 47, 29, 28, and 30 samples as positive, respectively, and the triple-target kit, kit F identified 29 samples as positive. Notably, some suspected positive cases were
Fig. 1. The relationship between concentration and Ct value of six RT-qPCR kits. Dot circle indicates abnormal results. (A) kit A; (B) kit B; (C) kit C; (D) kit D; (E) kit E; (F) kit F.

### Table 3
Demographic and clinical characteristics of the total cohort.

| Patients (N = 47) | Median age, years | Women | Confirmed cases of COVID-19 | Symptoms |
|-------------------|-------------------|-------|-----------------------------|----------|
|                   | 47 (16–80)        | 22    | 47                          | Fever and cough 37 |
|                   |                   |       |                             | Only fever 4 |
|                   |                   |       |                             | No fever and cough 6 |
|                   |                   |       |                             | Serious chronic disease |
|                   |                   |       |                             | Hypertension 3 |
|                   |                   |       |                             | Sampling timea, day 1–24 |
|                   |                   |       |                             | Total number of samples 57 |
|                   |                   |       |                             | Types of sample |
|                   |                   |       |                             | Induced sputum 49 |
|                   |                   |       |                             | Throat swabs 8 |

* Sampling time was from day 1 to day 24 after the onset of symptoms.

### Table 4
Classification of the samples according to their viral load.

| Classification        | Viral Load (copies/μL) | Number of Samples |
|-----------------------|------------------------|-------------------|
| Strongly positive     | > 1000                 | 4                 |
| Weakly positive 1     | 167–303                | 5                 |
| Weakly positive 2     | 5–80                   | 28                |
| Negative              | < 5                    | 20                |

### Table 5
Comparison of the results of six RT-qPCR kits for clinical samples testing.

|                   | A | B | C | D | E | F |
|-------------------|---|---|---|---|---|---|
| Positive          | 35| 47| 29| 28| 30| 29|
| Negative          | 22| 10| 28| 21| 21| 27|
| Suspected positive | 0 | 0 | 0 | 8 | 6 | 1 |
| Total             | 57| 57| 57| 57| 57| 57|
Our results were supported by a recent study. Chue et al. showed that the sensitivity of the N gene assay is sufficient for improving its accuracy because the results of different genes can be used for a single-target detection. Nevertheless, dual- or triple-gene detection may be more difficult and its sensitivity may significantly lower than that of a single-target detection kit (kit C) with the lowest detection sensitivity, which was 125 copies/mL and 4000 copies/mL, respectively. In addition, the dual-target kits, kits B and D, were found positive in 40 and 28 samples as positive, respectively. The single-target detection kits based on RT-qPCR from six companies were evaluated. Among the six kits, the dual-target kits, kits B and C had the highest detection sensitivity, which was 125 copies/mL and 4000 copies/mL, respectively. In addition, the dual-target kits, kits B and D had the highest and the lowest positive detection rate, which identified the lowest loading volume of the sample (2 µL). The performance of kits D, E, and F were comparable. However, the standards of the three kits are different. The kits D and E consider a sample as positive only when both the ORF1ab and N genes are positive. The kit F tests three target genes, so a sample is considered positive when both ORF1ab and N genes are positive or both ORF1ab and E genes are positive. These results suggested that the positive detection rate of a detection kit might be related to the loading volume of the sample.

### Table 6

Statistic of the single-gene-positive samples and multi-gene-positive samples.

|       | A   | B   | C   | D   | E   | F   |
|-------|-----|-----|-----|-----|-----|-----|
| Positive |     |     |     |     |     |     |
| O + N positive | 35  | 47  | 29  | 28  | 30  | 29  |
| O + E positive | N/A | 30  | 12  | 28  | 30  | 28  |
| Only O positive | 35  | 1   | 0   | 2   | 1   | 2   |
| Only N positive | N/A | 16  | 17  | 6   | 5   | 4   |
| Only E positive | N/A | N/A | N/A | N/A | N/A | 7   |

“O”: ORF1ab gene, “N”: N gene, “E”: E gene, “N/A”: Not Available.

reported by kits D, E, and F. Again, these results suggested that the number of targets did not directly affect the positive detection rate.

### 3.4. Analysis of the clinical test results based on the number of single-gene positive cases and multi-gene positive cases

We next calculated the samples that were positive for a single target gene (ORF1ab, N, or E gene) or multiple genes to further analyze the test results of the six RT-qPCR kits (Table 6). The kit A only tests for the ORF1ab gene, and is relatively sensitive to the ORF1ab gene with 35 samples were found positive. Among the 47 positive samples detected by the kit B, 17 samples were single-gene positive (16 were N gene positive and 1 was ORF1ab gene positive). According to the kit B’s instruction, a sample is considered positive when the ORF1ab gene or the N gene is positive. Also, the kit B required 20 µL of loading volume of the sample, which was the highest among the six RT-qPCR kits. Therefore, the kit B has the highest positive detection rate. Although kit C considers a sample positive when either the ORF1ab or N gene is positive, it only identified 29 positive samples. This might be related to the low loading volume of the sample (2 µL). The performance of kits D, E, and F were comparable. However, the standards of the three kits are different. The kits D and E consider a sample as positive only when both the ORF1ab and N genes are positive. The kit F tests three target genes, so a sample is considered positive when both ORF1ab and N genes are positive or both ORF1ab and E genes are positive. These results suggested that the positive detection rate of a detection kit might be related to the loading volume of the sample.

### 4. Discussion

Nucleic acid detection is the gold standard for the diagnosis of COVID-19, but its sensitivity may be low, resulting in false negatives. Therefore, it is necessary to evaluate the performance of different detection kits to figure out the cause of false-negative results. In this study, the performance of single-, dual-, triple-target SARS-CoV-2 detection kits based on RT-qPCR from six companies were evaluated. Among the six kits, the dual-target kits, kits B and C had the highest and the lowest detection sensitivity, which was 125 copies/mL and 4000 copies/mL, respectively. In addition, the dual-target kits, kits B and D had the highest and the lowest positive detection rate, which identified 47 and 28 samples as positive, respectively. The single-target detection kit (kit A) and triple-target detection kit (kit F) did not show better or worse performance. Therefore, these results showed that the performance of a kit was not directly related to the number of targets.

As the intrinsic interference and competition among primer pairs and probe in the same reaction, the design and optimization of an assay are more difficult and its sensitivity may significantly lower than that of single-target detection. Nevertheless, dual- or triple-gene detection may improve its accuracy because the results of different genes can be used for cross-validation. Our finding that only the N gene was detected in most samples indicates that the sensitivity of the N gene assay is sufficiently high for the detection of weakly positive clinical specimens. Our results were supported by a recent study. Chu et al. showed that the N gene could be recommended as a screening test of SARS-CoV-2 in RT-qPCR with the ORF1ab gene as a validation test [12].

The amount of sample loading in the assay affects detection sensitivity. Among the kits, kit B had the best performance, while the sensitivity of the kit C was the lowest. This may be related to their different amounts of sample loading, where kit B requires the highest amount (20 µL) while kit C requires the lowest amount (2 µL).

Although all the samples collected in this study were from patients with diagnosed infection of SARS-CoV-2, the samples of only 2 patients were collected at hospital admission, while the samples from the others were collected during the disease monitoring process. Thus, the patients included may not all be positive for SARS-CoV-2 at the time of sample collection. It has been reported that patients who were negative for the detection of nucleic acid for two consecutive days during the treatment later tested positive again [13]. This report and our findings suggest that multiple and multi-site sampling is essential, especially when making decisions regarding patient discharge. This decision should involve the evaluation of the nucleic acid detection results together with clinical symptoms and imaging findings.

With respect to quantitative performance, ddPCR is outstanding. It detects ORF1ab and N simultaneously, which improves accuracy. A recent study reported that the viral load is closely related to the intensity of lung injury and is also the key factor that determines the severity of disease, and a high viral load may be associated with an increased risk of fulminant myocarditis [14]. Therefore, using ddPCR to determine the copy number of the virus may be useful.

### 5. Conclusion

In summary, the number of targets in a detection kit is not a key factor affecting sensitivity, while the amount of sample loading may influence the performance of a detection kit. Meanwhile, ddPCR is excellent with absolute quantification and high sensitivity, and it may guide the development of diagnostic kits used for the diagnosis of COVID-19.

CRedit authorship contribution statement

Xiaomei Hu: Data curation, Formal analysis, Writing - original draft. Lingxian Zhu: Data curation, Formal analysis, Writing - original draft. Yijun Luo: Data curation, Formal analysis, Writing - original draft. Qinfei Zhao: Investigation, Validation, Visualization, Writing - original draft. Chianru Tan: Investigation, Validation, Visualization, Writing - original draft. Xiao Chen: Investigation, Validation, Visualization, Writing - original draft. Huijuan Zhang: Investigation, Validation, Visualization, Writing - original draft. Xiaojun Hu: Investigation, Validation, Visualization, Writing - original draft. Liping Lu: Investigation, Validation, Visualization, Writing - original draft. Yongwei Xiao: Investigation, Validation, Visualization, Writing - original draft. Shao Huang: Investigation, Validation, Visualization, Writing - original draft. Yingke He: Investigation, Validation, Visualization, Writing - original draft. Jacqueline Xi Ling Sim: Investigation, Validation, Visualization, Writing - original draft. Fang Wang: Funding acquisition, Validation. Yanke Peng: Validation, Visualization. Jigang Wang: Methodology, Project administration, Writing - review & editing. Yong Guo: Methodology, Project administration, Writing - review & editing. Tianyu Zhong: Methodology, Project administration, Writing - review & editing.

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.
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References

[1] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F. Zhan, X. Ma, D. Wang, W. Xu, G. Wu, G.F. Gao, W. Tan, A novel coronavirus from patients with pneumonia in China, 2019, N. Engl. J. Med. 382 (8) (2020) 727–733, https://doi.org/10.1056/NEJMoa2001017.

[2] World Health Organization: Coronavirus disease (COVID-2019) situation reports, https://apps.who.int/iris/bitstream/handle/10665/332089/nCoVsitrep13May2020-eng.pdf?sequence=1&isAllowed=y, 2020 (accessed 7 October 2020).

[3] R. Lu, X. Zhao, J. Li, P. Niu, B. Yang, H. Wu, W. Wang, H. Song, B. Huang, N. Zhu, Y. Bi, X. Ma, F. Zhan, L. Wang, T. Hu, H. Zhou, Z. Hu, W. Zhou, L. Zhao, J. Chen, Y. Meng, J. Wang, Y. Lin, J. Yuan, Z. Xie, J. Ma, W. Liu, B. Wang, W. Xu, E.C. Holmes, G.F. Gao, G. Wu, W. Chen, W. Shi, W. Tan, Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding, Lancet 395 (10224) (2020) 565–574, https://doi.org/10.1016/S0140-6736(20)30308-0.

[4] W. Guan, Z. Ni, Y. Hu, W. Liang, C. Ou, J. He, L. Liu, H. Shan, C. Lei, D.S.C. Hui, B. Du, L. Li, G. Zeng, K. Yuen, R. Chen, C. Tang, T. Wang, P. Chen, J. Xiang, S. Li, J. Wang, Z. Liang, Y. Peng, L. Wei, Y. Liu, Y. Hu, P. Peng, J. Wang, J. Liu, Z. Chen, G. Li, Z. Zhen, S. Qiu, J. Luo, C. Ye, S. Zhu, N. Zhong, Clinical Characteristics of Coronavirus Disease 2019 in China, N. Engl. J. Med. 382 (18) (2020) 1708–1720. https://doi.org/10.1056/NEJMoa200232.

[5] National Health Commission of the People’s Republic of China. Diagnosis and Treatment Protocol for COVID-19 (trial version 7), http://en.nhc.gov.cn/2020-03/29/c_78469.htm, 2020 (accessed 7 October 2020).

[6] V.M. Gorman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.Kw. Chu, Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Eurosurveillance 25 (3) (2020) 2000045, https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.

[7] X. Xie, Z. Zhong, W. Zhao, C. Zheng, F. Wang, J. Liu, Chest CT for Typical 2019-nCoV pneumonia: relationship to negative RT-PCR testing, Radiology 296 (2) (2020) E41–E45, https://doi.org/10.1148/radiol.2020200343.

[8] W. Zhao, Z. Zhong, X. Xie, Q. Yu, J. Liu, CT scans of patients with 2019 novel coronavirus (COVID-19) pneumonia, Theranostics 10 (10) (2020) 4606–4613, https://doi.org/10.7150/thno.45016.

[9] National Medical Products Administration of the People’s Republic of China, http://app1.nmpa.gov.cn/ (accessed 7 October 2020).

[10] [11] B. Vogelstein, K.W. Kinzler, Digital PCR, Proc. Natl. Acad. Sci. U.S.A. 96 (16) (1999) 9236–9241, https://doi.org/10.1073/pnas.96.16.9236.

[11] F. Yu, L. Yan, N. Wang, S. Yang, L. Wang, Y. Tang, G. Gao, S. Wang, C. Ma, R. Xie, F. Wang, C. Tan, L. Zhu, Y. Guo, F. Zhang, Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients, Clin. Infect. Dis. (2020) ciaa345, https://doi.org/10.1093/cid/ciaa345.

[12] D.K.W. Chu, Y. Pan, S.M.S. Cheng, K.P.Y. Hui, P. Krishnan, Y. Liu, D.Y.M. Ng, C.I.C. Wan, P. Yang, Q. Wang, M. Peiris, L.L.M. Poon, Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia, Clin. Chem. 66 (4) (2020) 549–555, https://doi.org/10.1093/clinchem/hva029.

[13] J. Lim, S. Jeon, H.Y. Shin, M.J. Kim, Y.M. Seong, W.J. Lee, K.W. Chee, Y.M. Kang, B. Lee, S.J. Park, Case of the index patient who caused tertiary transmission of COVID-19 infection in Korea: the application of lopinavir/ritonavir for the treatment of COVID-19 infected pneumonia monitored by quantitative RT-PCR, J. Korean. Med. Sci. 35 (6) (2020) e79, https://doi.org/10.3346/jkms.2020.35.e79.

[14] Y. Liu, Y. Yang, C. Zhang, F. Huang, F. Wang, J. Yuan, Z. Wang, J. Li, J. Li, C. Feng, Z. Zhang, L. Wang, L. Peng, L. Chen, Y. Qin, D. Zhao, S. Tan, L. Yin, J. Xu, C. Zhou, C. Jiang, L. Liu, Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury, Sci. China Life Sci. 63 (3) (2020) 364–374, https://doi.org/10.1007/s11427-020-1643-8.