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Digital PCR is a sensitive new technique for SARS-CoV-2 detection in clinical applications

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ABSTRACT

The global coronavirus disease 2019 (COVID-19) pandemic has posed great challenges in people’s daily lives. Highly sensitive laboratory techniques played a critical role in clinical COVID-19 diagnosis and management. In this study the feasibility of using a new digital PCR-based detection assay for clinical COVID-19 diagnosis was investigated by comparing its performance with that of RT-PCR. Clinical patient samples and samples obtained from potentially contaminated environments were analyzed. The study included 10 patients with confirmed COVID-19 diagnoses, 32 validated samples of various types derived from different clinical timepoints and sites, and 148 environmentally derived samples. SARS-CoV-2 nucleic acids were more readily detected in respiratory tract samples (35.0%). In analyses of environmentally derived samples, the positivity rate of air samples was higher than that of surface samples, probably due to differences in virus concentrations. Digital PCR detected SARS-CoV-2 in several samples that had previously been deemed negative, including 3 patient-derived samples and 5 environmentally derived samples. In this study digital PCR exhibited higher sensitivity than conventional RT-PCR, suggesting that it may be a useful new method for clinical SARS-CoV-2 detection. Improvement of SARS-CoV-2 detection would substantially reduce the rates of false-negative COVID-19 test results, in particular those pertaining to asymptomatic carriers.

1. Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). By 20 April 2020 there had been more than 2,360,000 confirmed COVID-19 cases worldwide, and over 165,000 deaths. It has been suggested that the total number of reported SARS-CoV-2 infections is an underestimate of the actual number of infections, due to mild and asymptomatic cases [1]. COVID-19 patients exhibit various clinical courses, but the most common symptoms are fever (43.8% on admission and 88.7% during hospitalization) and coughing (67.8%) [2].

Laboratory tests and computed tomography (CT) scans have been used in clinical practice to diagnose COVID-19 and monitor treatment responses [3], and more recently multiple SARS-CoV-2 nucleic acid detection kits have been developed. To date real-time reverse-transcriptase (RT) PCR is the most reliable technology for the detection and quantification of target nucleic acid fragments. It constitutes a rapid and accurate method of viral nucleic acid detection, and it has been widely utilized for viral DNA detection in both clinical and environmentally derived samples.

The most substantial aspect of real-time RT-PCR is that it facilitates monitoring of the amplification of the target DNA sequence that is reverse-transcribed from RNA, then mathematical quantification of the starting material after background correction [4]. For assays designed for diagnostic applications, viral nucleic acid in a patient-derived sample is often the detection target. Notably however, it has been reported that in clinical practice the false-negative rate of real-time RT-PCR-based assays can be as high as 20% [5]. This potential false-negative rate raises
a general concern given its diagnostic purpose, and may result in substantial clinical consequences [5]. There is a need for improvement of detection assays used for COVID-19 laboratory diagnosis.

Digital PCR is an emerging technology designed to achieve absolute quantification after real-time PCR, and it has been successfully applied in various fields, including clinical laboratory diagnosis [6–9]. In the current study the use of a novel digital PCR assay to detect SARS-CoV-2 in both clinical patient-derived samples and environmentally derived samples was investigated, with the ultimate aim of reducing the rate of false negative results.

2. Materials and methods

2.1. Study population

All SARS-CoV-2 tests were performed at a genetic diagnosis center from January 2020 to April 2020. Ten patients diagnosed with COVID-19 in accordance with the World Health Organization Interim Guidelines were included in the study [10]. The clinical characteristics of the 10 patients are shown in Table S1. The WHO Interim Guidelines stipulate that a relevant epidemiological history and any two of the following clinical manifestations and pathogenic evidence are indicative of potential COVID-19:

- Within 14 days before the onset of symptoms the patient has engaged in tourism or residence in Wuhan or its surrounding areas, or other communities with confirmed cases; (2) Within 14 days before the onset of symptoms the patient has been in contact with confirmed cases of COVID-19; (3) Within 14 days before the onset of symptoms the patient has been in contact with suspected cases (patients with fever or respiratory symptoms) from Wuhan or its surrounding areas, or other communities with confirmed cases; (4) With regard to aggregation, within 14 days before the onset of symptoms one confirmed case was detected in an enclosed environment (such as a domestic premises, a construction site, or an office) and one or more cases of fever or respiratory tract infection were detected at the same time.

Clinical manifestations: (1) Fever and/or respiratory symptoms; (2) Imaging indicates multiple motting and interstitial changes in lung consolidation; (3) In the early stage of the disease the total number of leukocytes was normal or decreased, or the lymphocyte count was decreased.

Pathogenic evidence: Nucleic acid test (real-time RT-PCR) was used to detect SARS-CoV-2 in the respiratory tract. In the present study SARS-CoV-2 nucleic acid was detected in nasopharyngeal swabs from all patients except patient 9. In patient 9 SARS-CoV-2 nucleic acid was detected in an oropharyngeal swab sample.

2.2. Sampling and sample processing

Thirty-two patient samples including nasopharyngeal swabs, throat swabs, oropharyngeal swabs, phlegm, plasma/blood, and eye conjunctiva were collected at multiple timepoints during the disease course, and tested for the presence of SARS-CoV-2 via RT-PCR. RT-PCR cycle threshold values were determined. Plasma/blood was taken from the cubital vein.

A total of 148 environmentally derived samples were collected from potentially contaminated areas. Two isolation ward areas were included in the study, and each area included a nursing station. The environmental samples included air samples and surface samples, and the sample collection points utilized have been described previously [5,11]. Environmental monitoring was conducted in accordance with the hospital sanitation standards [12]. All air samples were collected by natural sedimentation and a microbial air sampler (MAS-100 ECO) with the air stream set to 500 L (Merck, Germany). Environmental surfaces were sampled via swabs. RT–PCR cycle threshold values were determined. Sample collection and environmental sampling details are shown in Table S2.

2.3. Primer and probe sequences

The expected amplicon sizes of the ORF1ab and N gene assays were 132 bp and 110 bp. All primers and probes were purchased from Integrated DNA Technologies. It was retrospectively confirmed that the primer and probe sequences were perfectly matched with SARS-CoV-2 genome sequences from the Global Initiative on Sharing All Influenza Data (https://www.gisaid.org/; accession numbers EPI_ISL_402119, EPI_ISL_402120, EPI_ISL_402121, EPI_ISL_402123, and EPI_ISL_402124; accessed 12 January 2020).

2.4. RNA extraction

All samples were inactivated in 600 μl guanidine hydrochloride buffer, centrifuged at 12,000 g for 10 min, then incubated for 30 min. After discarding the supernatant, 50 μl of RNA release agent (Shanghai, Hunan, China) was added. The samples were then mixed and incubated for 10 min, and the extracted RNA was used for RT-PCR and digital PCR.

2.5. Real-time RT-PCR

Real-time RT-PCRs were conducted in a 50-μl reaction containing 20 μl RNA and 30 μl 1× reaction buffer. The reaction buffer consisted of 26 μl SARS-CoV-2 PCR mix that contained primers (4.6%), probes (1.2%), dNTPs (3.9%), MgCl₂ (0.8%), RNasin (0.5%), and PCR buffer (89.1%) (Shengxiang, Hunan, China), and 4 μl SARS-CoV-2 PCR enzyme mix containing RT enzyme (62.5%) and Taq enzyme (37.5%) (Zhijiang, Shanghai, China). Thermal cycling was performed at 50 °C for 30 min, 95 °C for 1 min, then 45 cycles of 95 °C for 15 s and 60 °C for 30 s. A Hongshi SLAN 96 S instrument (Hongshi, Shanghai, China) was used. When the quantitative PCR cycle threshold value was ≤ 43 the sample was considered positive.

2.6. Digital PCR

All digital PCR procedures were performed using the Droplet Digital PCR System (Changchun Technical Biotechnology Co., Ltd. Changchun, China) and the SARS-CoV-2 Nucleic Acid Detection Kit (Shanghai Righong Biotechnology Co., Ltd. Shanghai, China) in accordance with the manufacturer’s instructions. The pre-amplification mixture was prepared by using 12.50 μl CS-mix, 1.25 μl CS-SARS-COV-2, 1.25 μl CS-enzyme (SARS-CoV-2 Nucleic Acid Detection Kit, Shanghai Righong Biotechnology Co., Ltd.). Ten microliters of nucleic acid was added to the pre-amplification mixture, which contained 25 μl of each reaction system. Pre-amplification was performed at 55 °C for 15 min, followed by 95 °C for 30 s, then 8 cycles of 95 °C for 10 s and 60 °C for 45 s, and a final extension at 12 °C for 5 min.

Droplet preparation was conducted using a droplet generator (Droplet Digital PCR System) and a droplet generator chip. The droplet was then transferred to an RNAse-free PCR tube for PCR amplification. The amplification mixture was prepared using 4 μl ddPCR-A or ddPCR-B
and 10 μL ddPCR MIX3 (SARS-CoV-2 Nucleic acid Detection Kit, Shanghai Rightongene Biotechnology Co., Ltd). Amplification was initiated at 95 °C for 10 min, followed by 40 cycles of 94 °C for 15 s and 58 °C for 60 s, then final extension at 98 °C for 10 min.

After amplification a biochip reader (Droplet Digital PCR System) was used to analyze fluorescence, and a two-color optical detection system was used for image scanning and analysis of each droplet in the sample. Droplets with fluorescent signals were considered positive, and droplets without fluorescent signals were considered negative. Numbers of positive and negative droplets and proportions of positive droplets were calculated. Lastly, based on the Poisson distribution principle and the proportion of positive droplets, analysis software was used to calculate the concentration or copy number of target molecules.

3. Results

3.1. Characteristics of COVID-19-positive patients

Details of the 10 patients diagnosed with COVID-19 included in the current study are shown in Table 1. The mean age of the initial patient cohort was 56.9 years (range 25–79 years) and 36.4% were male. The patients were clinically classified as mild, moderate, severe, or critical based on C-reactive protein. In total 72.7% of patients (n = 10) had more than one listed symptom. The most common clinical symptoms were coughing (54.6%), diarrhea (36.4%), and fever (27.3%). Bilateral pneumonia was evident in CT scans of 81.8% of patients, and unilateral pneumonia was evident in CT scans of 9.1% of patients. Contact tracing indicated that 81.8% of the patients had been in contact with either confirmed COVID-19 cases or people associated with Wuhan.

Table 1

| Characteristics of COVID-19-positive patients. | Patients(n = 10) |
|---|---|
| **Age** | 56.9 ± 18.12 |
| Mean (SD) | 56.9 ± 18.12 |
| 20–30 | 1 |
| 40–50 | 4 |
| 50–60 | 1 |
| 70–80 | 4 |
| **Sex** | 54.55% |
| Female | 4 |
| Male | 6 |
| **Clinical classification** | 18.18% |
| Mild type | 3 |
| Moderate type | 4 |
| Severe type | 1 |
| Critically ill type | 2 |
| **Temperature** | 18.18% |
| 36.2°C–37.2°C | 6 |
| 37.3°C–38.4°C | 1 |
| 38.5°C–39°C | 2 |
| 39.1°C–41°C | 1 |
| **Signs and symptoms at admission** | 9.09% |
| Fever | 3 |
| Cough | 6 |
| Shortness of breath | 1 |
| Muscle ache | 1 |
| Sore throat | 2 |
| Diarrhea | 4 |
| More than one sign or symptom | 8 |
| Chest x-ray and CT finding | 9 |
| Bilateral pneumonia | 81.82% |
| Unilateral pneumonia | 1 |
| No abnormal density shadow | 0.00% |
| Epidemiological history | 9 |
| Contacts with confirmed cases of COVID-19 or Wuhan related people | 81.82% |
| No contract history | 1 |

3.2. Positivity rate in respiratory tract samples from confirmed COVID-19 patients

SARS-CoV-2 nucleic acid sequences were detected in all clinical patient samples (respiratory tract samples including nasopharyngeal and oropharyngeal swabs). To investigate associations between positivity rate and sample type, additional testing of 32 samples collected from respiratory and non–respiratory sites in 4 confirmed COVID-19 patients at different timepoints during hospitalization was conducted, and the results are shown in Table 2. Different positivity rates were associated with different sample types (Table 3). SARS-CoV-2 was more frequently detected in respiratory tract-derived samples (35.0%) than in non-respiratory tract-derived samples (0.0%). The positivity rate was highest in phlegm samples (66.7%), and the detection rate in oropharyngeal swabs (42.9%) was higher than that in nasopharyngeal swabs (28.6%).

3.3. Positivity rates in environmental samples

Of a total of 148 environmentally derived samples collected from potentially contaminated areas, 2 (1.4%) tested positive for SARS-CoV-2 in real-time RT-PCR assays. Compared to samples collected from...
potentially contaminated surfaces, a higher positivity rate was evident in air samples (1/28, 3.6%). The results suggest that SARS-CoV-2 detection rate may be affected by the pathogen concentration in the environment (Table 4).

### 3.4. Comparative sensitivity of digital PCR for SARS-CoV-2 detection

A novel digital PCR-based assay was developed to improve the accuracy of laboratory COVID-19 diagnosis, and all samples previously analyzed via real-time RT-PCR were re-analyzed using that digital PCR-based assay. All samples that tested positive in real-time RT-PCR assays also tested positive in digital PCR assays. Eight additional samples, including 3 clinical patient-derived samples and 5 samples from potentially contaminated environments only tested positive in digital PCR assays (Table 5). These 3 patient samples were derived from an oropharyngeal swab (from patient 7), blood/plasma (patient 8), and a nasopharyngeal swab (patient 9). The results suggest higher sensitivity of the digital PCR-based assay for SARS-CoV-2 detection in clinical applications. The application of digital PCR for SARS-CoV-2 detection may play a critical role in diminishing the false-negative rate of diagnostic COVID-19 laboratory tests.

### 4. Discussion

Numerous clinical symptoms have been reported in COVID-19 patients, and the clinical course can vary from asymptomatic to critical [2]. The most common symptoms of COVID-19 are reportedly fever, coughing, and a sore throat [2,11]. COVID-19 patients can also present symptoms listed was evident in 72.7% of the patients. CT scans depicted clinical features of bilateral pneumonia in 81.9% of patients and unilateral pneumonia in 9.1% of patients.

As in some previously reported studies [11], SARS-CoV-2 nucleic acid sequences were more frequently detected in respiratory tract-derived samples (35.0%) than in non-respiratory tract-derived samples (0.0%). The mucosa of the conjunctiva and upper respiratory tract are connected by the nasolacrimal duct, share the same SARS-CoV-2 entry receptor on cell membranes (angiotensin-converting enzyme 2), and may act as a transmission route for SARS-CoV-2 infection [19]. Notably however, no SARS-CoV-2 nucleic acid sequences were detected in eye conjunctiva in the current study. This may be related to the fact that no eye infection symptoms were evident in any of the patients in the study. SARS-CoV-2 RNA has been isolated from serum from SARS-CoV-2-infected patients [20], but no SARS-CoV-2 nucleic acid sequences were detected in plasma/blood samples in the current study. This may be because the concentration of SARS–CoV-2 nucleic acid in these samples was below the detection limits of the tests.

In a recent study the SARS-CoV-2 nucleic acid detection threshold concentration of real-time RT-PCR was reportedly 1 × 10^3 copies/mL, which is considered to constitute inadequate sensitivity and insufficient stability [21]. In another report the false-negative rate of SARS-CoV-2 real-time RT-PCR was approximately 20%, and this led to serious consequences [5]. Chest CT was once considered better than real-time RT-PCR for COVID-19 screening, comprehensive evaluation, and follow-up in epidemic areas [21]. Given the need for timely and accurate COVID-19 diagnosis, it has become necessary to minimize possible causes of false-negative nucleic acid-based test results, or develop a new more sensitive nucleic acid detection-based assay.

In the current study, of all respiratory tract sample types the detection rate in phlegm (66.7%) was the highest, and the detection rate in nasopharyngeal swabs (28.6%) was higher than that in oropharyngeal swabs (42.9%). This suggests that detection of SARS-CoV-2 is influenced by sample type. The rate of detection was greater in lower respiratory tract-derived samples than in upper respiratory tract-derived samples. Due to practical considerations, procedural factors, and comparative patient intolerance there are many difficulties associated with lower respiratory tract sampling. It has also been reported that nasopharyngeal aspirate had a higher positivity rate within 2 weeks of symptom onset, whereas combined nasal and oropharyngeal swabs were the least harmful to medical staff during sampling [22]. With regard to the environmental samples in the current study, the positivity rate of air samples was higher than that of surface samples. Environmental sampling results are influenced by the concentration of virus in the samples.

The low detection rate of real-time RT-PCR is associated with many factors including sampling procedures, nucleic acid quality, specimen source (upper or lower respiratory tract), and sampling timing (different periods of disease development). The quality of extracted nucleic acid has a strong influence on the outcomes of real-time RT-PCR. The template volume can be increased to raise the sensitivity of detection, and reagents containing guanidine salt can be used to inactivate virus as well as protect RNA [23]. To improve the positivity rate if lower respiratory tract specimens are unavailable, stool and blood samples can also be used at later stages of illness [23]. Nucleic acid tests are based on the concentration of virus, and serological tests using proteins can be utilized to supplement nucleic acid tests, enabling clinicians to track both sick and recovered patients, providing a better estimate of total SARS-CoV-2 infections [3].

Digital PCR has higher sensitivity and accuracy than standard real-time RT-PCR, and is developing rapidly and being widely applied in clinical microbiology, in fields such as drug resistance mutations in hepatitis C virus, Staphylococcus aureus, and influenza A virus [24–26]. To prevent false-negative SARS-CoV-2 nucleic acid-based test results, and develop a new sensitive detection assay, we evaluated the performance of real-time RT-PCR and digital PCR for detecting SARS-CoV-2 nucleic acid in clinical patient-derived samples and environmentally derived samples.

A newly developed digital PCR assay was performed with samples that had previously been tested using real-time RT-PCR. All the real-time RT-PCR positive samples also tested positive in the digital PCR assay. Strikingly, digital PCR detected SARS-CoV-2 nucleic acids in several samples that had previously tested negative via real-time RT-PCR, including 3 patient-derived samples and 5 environmentally derived samples. These results suggest that digital PCR is a more efficient and sensitive method for SARS-CoV-2 detection than real-time RT-PCR.

In some recent studies reactivation of SARS-CoV-2 was evident in

### Table 3

| Sample type             | Total No. | Positive | Positive rate |
|-------------------------|-----------|----------|---------------|
| Respiratory tract sample| 20        | 7        | 35.00%        |
| Non-respiratory sample  | 12        | 0        | 0.00%         |
| Plasma/blood            | 8         | 0        | 0.00%         |
| Total                   | 32        | 7        | 21.88%        |

### Table 4

| Monitoring type         | Total No. | Positive | Positive rate |
|-------------------------|-----------|----------|---------------|
| Air monitoring samples  | 28        | 1        | 3.57%         |
| Surface monitoring      | 120       | 1        | 0.83%         |
| Total                   | 148       | 2        | 1.35%         |
some recovered patients, and there were no specific clinical characteristics to distinguish them [27,28]. In another study a recovered COVID-19 patient returned to being SARS-CoV-2-positive, raising concern about the present standards for patient discharge [29]. These observations indicate that a proportion of recovered patients may still be virus carriers. Stricter patient discharge standards and more sensitive detection assays are required to prevent the spread of SARS-CoV-2. Digital PCR can serve as a more sensitive nucleic acid detection assay. It can detect viral nucleic acid present at low concentrations. Thus, it has the potential to improve the detection rate in asymptomatic carriers.

Digital PCR constitutes a fundamentally different approach to quantifying the number of DNA molecules in a sample, and quantification is achieved without the need for PCR cycle threshold values and standard curves [30]. In conclusion, digital PCR can be used in clinical applications as a new sensitive nucleic acid detection assay for SARS-CoV-2 in the future. Its use will reduce the rate of false-negative results, and improve the detection rate of asymptomatic carriers.

**Declaration of Competing Interest**

The authors declared that there is no conflict of interest.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2020.10.032.

**Table 5**

Comparison of digital PCR results and real-time RT-PCR results in negative real-time RT-PCR detected samples.

| Case/area | Sample type | real-time RT-PCR result | Digital PCR result |
|-----------|-------------|-------------------------|--------------------|
| Case 7    | Oropharyngeal swabs | – | – |
| Case 8    | Blood/plasma | – | – |
| Case 9    | Nasopharyngeal swab | – | – |
| Isolation ward | Air | – | – |
| Isolation ward | General subject surface of the refuse storage area near the nurse station | – | – |
| Isolation | Outer side of the toilet | – | – |
| Isolation | Inner side of the sink in the toilet | – | – |
| Isolation | Outer side of the sink in the toilet | – | – |

**References**

[1] T. Kobayashi, S.M. Jung, N.M. Linton, R. Kinoshita, K. Hayashi, T. Miyama, et al., Communicating the Risk of Death from Novel Coronavirus Disease (COVID-19), J. Clin. Med. 9 (2) (2020).

[2] W.J. Guan, Z.Y. Ni, Y. Hu, W.H. Liang, C.Q. Ou, J.X. He, et al., Clinical Characteristics of Coronavirus Disease 2019 in China, New England J. Medicine (2020).

[3] B. Udugama, P. Kadiheres, H.N. Kozlovski, A. Malekjahani, M. Osborne, V.Y. C. Li, et al., Diagnosing COVID-19: The Disease and Tools for Detection, ACS Nano (2020).

[4] V.M. Cormorn, O. Landt, M. Kainer, R. Molenkamp, A. Meijer, D.K. Chu, et al., Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro surveillance : bulletin European sur les maladies transmissibles, European Communicable Disease Bull. 25 (2020).

[5] Y. Jiang, H. Wang, Y. Chen, J. He, L. Chen, Y. Liu, et al., Clinical Data on Hospital Environmental Hygiene Monitoring and Medical Staff Protection during the Coronavirus Disease 2019, Outbreak. 2020 (02) (2020), 25.20028043.

[6] Lin J, Su G, Su W, Zhou C. [Progress in digital PCR technology and application]. Sheng wu gong cheng xue bao — Chinese journal of biotechnology. 2017;33(2):170-7.

[7] Zulak KG, Cox BA, Tucker MA, Oliver RP, Lopez-Ruiz FJ. Improved Detection and Monitoring of Fungal Resistance in Blumeria graminis f. sp. hordei With High-Throughput Genotype Quantification by Digital PCR. Frontiers in microbiology. 2018;9:706.

[8] F. Martínez-Hernández, I. García-Heredia, M. Llesesma, L. Laestre-Carbàlia, J. Martínez-Martínez, M. Martínez-García, Droplet Digital PCR for Estimating Absolute Abundances of Widespread Pelagibacter Viruses, Front. Microbiol. 10 (2019) 1226.

[9] L.L. Ren, Y.M. Wang, Z.Q. Wu, Z.C. Xiang, L. Guo, T. Xu, et al., Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study, Chin. Med. J. (2020).

[10] W. Wang, J. Tang, F. Wei, Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. Journal of medical virology. 92 (4) (2020), 441-7.

[11] J.F. Huggett, S. Cowen, C.A. Foy, Considerations for digital PCR as an accurate molecular diagnostic tool, Clin. Chem. 61 (1) (2015) 79-86.

[12] Y. Li, J. Shi, J. Xia, J. Duan, L. Chen, Y. Yu, et al., Asymptomatic and Symptomatic Patients With Non-severe Coronavirus Disease (COVID-19) Have Similar Clinical Features and Virolological Courses: A Retrospective Single Center Study, Front. Microbiol. 11 (2020) 1570.

[13] Z. Liu, C.B. Sun, Conjunctiva is not a preferred gateway of entry for SARS-CoV-2 to infect respiratory tract, J. Med. Virol. (2020).

[14] D. Wang, B. Hu, C. Hu, F. Zhu, X. Liu, J. Zhang, et al., Asymptomatic and Symptomatic Patients With Non-severe Coronavirus Disease (COVID-19) Have Similar Clinical Features and Virolological Courses: A Retrospective Single Center Study, Front. Microbiol. 11 (2020) 1570.

[15] B.E. Young, S.W.X. Ong, S. Kalimuddin, J.G. Low, S.Y. Tan, J. Loh, et al., Communicating the Risk of Death from Novel Coronavirus Disease (COVID-19), Jama, 2020.

[16] S. Sullivan, et al., Detection of Rare Drug Resistance Mutations by Digital PCR Using a Riboflavin and Ultraviolet Light-based Photochemical Treatment, Vox Sang. 110 (2020) 437-47.

[17] S.D. Keil, I. Ragan, S. Yonemura, L. Hartson, N.K. Dart, R. Bowen, Inactivation of severe acute respiratory syndrome coronavirus 2 in plasma and platelet products using a riboflavin and ultraviolet light-based photochemical treatment, Vox Sang. 20 (5) (2016), 437-47.

[18] W. Wang, J. Tang, F. Wei, Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. Journal of medical virology. 92 (4) (2020), 441-7.

[19] J. Li, Z. Cao, P. Jiang, Q. Wang, Y. Sun, et al., Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study, Chin. Med. J. (2020).

[20] L.L. Ren, Y.M. Wang, Z.Q. Wu, Z.C. Xiang, L. Guo, T. Xu, et al., Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study, Chin. Med. J. (2020).

[21] Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of Chest CT and RT-PCR Testing in Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. Radiology, 2020:206642.

[22] Be B, Fan C, Pan Y, Ding R, Hu RX, Xiang ML. [Which sampling method for the upper respiratory tract specimen should be taken to diagnose patient with COVID-19?]. Zhonghua yuan yu ba gui ji chuan zong yi yan jiu zazhi. 2020;1(3):102-6.

[23] Yan Y, Chang L, Wang L. Laboratory testing of SARS-CoV, MERS-CoV, and SARS-CoV-2 (2019-nCoV): Current status, challenges, and countermeasures. Reviews in medical virology. 2020:22106.

[24] A.S. Whal, C.A. Bushell, P.R. Grant, S. Cowen, I. Gutiérrez-Aguirre, D. M. O’Sullivan, et al., Detection of Rare Drug Resistance Mutations by Digital PCR in a Human Influenza A Virus Model System and Clinical Samples, J. Clin. Microbiol. 54 (2) (2016) 392-400.

[25] J.F. Huggett, S. Cowen, C.A. Foy, Considerations for digital PCR as an accurate molecular diagnostic tool, Clin. Chem. 61 (1) (2015) 79-88.

[26] M. Vynck, W. Trypsteen, O. Thas, L. Vandekerckhove, W. De Spiegelaere, The Future of Digital Polymerase Chain Reaction in Virology, Molecular Diagnosis Therapy 20 (5) (2016), 437-47.
[27] G. Ye, Z. Pan, Y. Pan, Q. Deng, L. Chen, J. Li, et al., Clinical characteristics of severe acute respiratory syndrome coronavirus 2 reactivation, J. Infection 80 (5) (2020) e14-e7.

[28] L. Lan, D. Xu, G. Ye, C. Xia, S. Wang, Y. Li, et al., Positive RT-PCR Test Results in Patients Recovered From COVID-19, JAMA 323 (15) (2020), 1502-3.

[29] Zhang JF, Yan K, Ye HH, Lin J, Zheng JJ, Cai T. SARS-CoV-2 turned positive in a discharged patient with COVID-19 arouses concern regarding the present standard for discharge. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2020.

[30] J. Kuypers, K.R. Jerome, Applications of Digital PCR for Clinical Microbiology, J. Clin. Microbiol. 55 (6) (2017), 1621-8.