ddPCR: a more sensitive and accurate tool for SARS-CoV-2 detection in low viral load specimens

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Abstract

Background: Real-Time PCR (RT-PCR) is widely used as the gold standard for clinical detection of SARS-CoV-2. However, due to the low viral load in patient throat and the limitation of RT-PCR, significant numbers of false negative reports are inevitable, which should not be ignored.

Methods: We explored the feasibility of droplet digital PCR (ddPCR) to detect SARS-CoV-2 from 57 clinical pharyngeal swab samples and compared with RT-PCR in terms of the sensitivity and accuracy. Among 57 samples, all of which were reported as negative nucleic acid by officially approved clinical RT-PCR detection, 43 samples were collected from suspected patients with fever in clinic, and 14 were from supposed convalescents who were about to discharge after treatment. The experiment was double-blind.

Results: The lower limit of detection of the optimized ddPCR is at least 500 times lower than that of RT-PCR. The overall accuracy of ddPCR for clinical detection is 94.3 %. 33 out of 35 negative pharyngeal swab samples checked by RT-PCR were correctly judged by ddPCR based on the follow-up investigation. In addition, 9 out of 14 (64.2 %) supposed convalescents with negative nucleic acid test twice by RT-PCR were positive by ddPCR detection.

Conclusions: ddPCR shows superiority for clinical detection of SARS-CoV-2 to reduce the false negatives, which could be a powerful complement to the current standard RT-PCR. Before the ddPCR to be approved for diagnosis, the current clinical practice that the convalescent continues to be quarantined for 2 weeks is reasonable and necessary.

Key words: SARS-CoV-2; droplet digital PCR; RT-PCR; clinical detection

Introduction

The recent outbreak of coronavirus disease 2019 (COVID-19) caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses a great threat to public health all over the world.[1,2] On February 28, 2020, the world health organization (WHO) has upgraded the global risk level of this viral pneumonia from "high" to "very high". According to WHO and Chinese Center for Disease Control and Prevention (CDC), the current gold standard for the diagnosis of SARS-CoV-2 infection is based on the real-time fluorescent quantitative PCR (RT-PCR), which means that the nucleic acid of SARS-CoV-2 could be detected in patient specimens using RT-PCR.[3,4] However, the disadvantages of insufficient detection of RT-PCR are more and more prominent, especially the problem of detection dynamic range in the clinical application. At present, it has been found in clinical practice that some patients had fever, and chest CT showed symptoms of suspected viral pneumonia such as lower lobe lesions of the lungs, but the nucleic acid test of pharyngeal swab did not show positive results until 5-6 days after the onset of viral pneumonia. It was estimated that only 30 %-60 % positive results can be obtained among COVID-19 patients that further confirmed by chest CT.[5] This might be explained by the relatively low viral load in the throat of patients and the sensitivity limitation of RT-PCR technology, which inevitably produced the false negatives during the clinical diagnosis, leading to a potential risk of viral transmission. Besides, supposed convalescent, who is about to discharge, also need multiple tests with negative results for confirmation. Therefore, it is a pressing needs for a more sensitive and accurate detection method for the pathogenic detection.

Digital PCR is based on the principles of limited dilution, end-point PCR, and Poisson statistics, with absolute quantification as its heart.[6] It has broader dynamic range without external interference and robustness to variations in PCR efficiency. [7–9] In 2011, Hindson developed the droplet digital PCR (ddPCR) technology based on traditional digital PCR.[10] The reaction mixture can be divided into tens of thousands of nanodroplets during the process. These vast and highly consistent oil droplets

substantially improve the detection dynamic range and accuracy of digital PCR in a low-cost and practical format.[11] In recent years, this technology has been widely used, such as analysis of absolute viral load from clinical samples, analysis of gene copy number variation, rare allele detection, gene expression, microRNA analysis and genome edit detection *et al*.[12,13,14,15] Here, taking the advantages of ddPCR, we optimized the preparation of pharyngeal swab samples, and develop a workflow of ddPCR to detect SARS-CoV-2 using Chinese CDC approved primer and probe sets. Based on the results of this optimized ddPCR system, we showed that the overall accuracy of the ddPCR for clinical pathogen detection is 94.3 %, and 64.2 % of supposed convalescents with two consecutive negative nucleic acid tests by RT-PCR still carry SARS-CoV-2.

Materials and methods

Ethics statement

This study was approved by the Ethics Committee of the Renmin Hospital and Zhongnan Hospital of Wuhan University. The analysis was performed on existing samples collected during standard diagnostic tests, posing no extra burden to patients, as described previously.[2]

Specimen collection and RNA extraction

Pharyngeal swab samples were obtained from clinical suspected patients with fever or rehabilitation quasi-discharged patients of COVID-19 at Renmin Hospital and Zhongnan Hospital of Wuhan University according to the interim guidance of WHO. Pharyngeal swabs were soaked in 500 μl PBS and vortexed with diameter of 3 mm beads (Novastar, China) for 15 seconds immediately. Total RNA was extracted from the supernatant using QIAamp viral RNA mini kit (Qiagen) following manufacturer's instruction. First strand cDNA was synthesized using PrimeScript RT Master Mix (TakaRa) with random primer and oligo dT primer.

Primers and probes

The primers and probes targeted the ORF1ab and N of SARS-CoV-2 according to Chinese CDC. Target 1 (ORF1ab), forward: 5'-CCCTGTGGGTTTTACACTTAA-3', reverse: 5'-ACGATTGTGCATCAGCTGA-3', probe: 5'-FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3';
Target 2 (N), forward: 5'-GGGGAACTTCTCCTGCTAGAAT-3',
reverse: 5'-CAGACATTTTGCTCTCAAGCTG-3',
probe: 5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3'.[16]

Droplet Digital PCR workflow

All the procedure follow the manufacture instructions of the QX200 Droplet Digital PCR System using supermix for probe (no dUTP) (Bio-Rad). Briefly, the TaqMan PCR reaction mixture was assembled from a 2× supermix for probe (no dUTP) (Bio-Rad), 20× primer and probes (final concentrations of 900 and 250 nM, respectively) and template (variable volume) in a final volume of 20 μl. Twenty microliters of each reaction mix was converted to droplets with the QX200 droplet generator (Bio-Rad). Droplet-partitioned samples were then transferred to a 96-well plate, sealed and cycled in a T100 Thermal Cycler (Bio-Rad) under the following cycling protocol: 95 ˚C for 10 min (DNA polymerase activation), followed by 40 cycles of 94 ˚C for 30 s (denaturation) and 60 ˚C for 1 min (annealing) followed by an infinite 4-degree hold. The cycled plate was then transferred and read in the FAM channels using the QX200 reader (Bio-Rad).

RT-PCR

The primers and probes used in ddPCR are also used in RT-PCR. A 30-μl reaction was set up containing 10 μl of RNA, 18.5 μl of reaction buffer provided with the one step RT-PCR system and 1.5 μl enzyme mix (BGI BIOTECHNOLOGY). Thermal cycling was performed at 50 °C for 20 min for reverse transcription, followed by 95°C for 10 min and then 40 cycles of 95 °C for 15 s, 60 °C for 30 s in BIO-RAD CFX96 Touch RT-PCR system.

148

149 **Data statistical analysis**

150 Analysis of the ddPCR data was performed with Quanta Soft analysis software
151 v.1.7.4.0917 (Bio-Rad) that accompanied the droplet reader calculate the
152 concentration of the target DNA sequences, along with their Poisson-based 95 %
153 confidence intervals. The positive populations for each primer/probe are identified
154 using positive and negative controls with single (i.e., not multiplexed) primer–probe
155 sets. The concentration reported by QuantaSoft equals copies of template per
156 microliter of the final 1× ddPCR reaction, which was also used in all the results. In
157 addition, plots of linear regression were conducted with GraphPad Prism 7.00, and
158 probit analysis for lower limit of detection (LLoD) was conducted with StatsDirect
159 software v3.2.9. Lower limit of quantitation (LLoQ) and LLoD were defined as the
160 lowest concentration at which 95 % and 50 % of positive samples were detected,
161 respectively.

162

163 **Results**

164 **Comparison of the lower limit between ddPCR and the standard RT-PCR**

165 Using a manual threshold to define positivity, 9 % of negative controls (3/32) were
166 scored as positive due to one single positive droplet (data not shown). The presence of
167 two positive droplets or more was not observed for negative controls. Serial dilutions
168 of a positive control DNA fragment of SARS-CoV-2 were tested with primers/probe
169 sets targeting ORF1ab and N of SARS-CoV-2, respectively for ddPCR. It shows good
170 linearity (R2: 0.9932 and 0.9824, respectively) (Fig. 1A and 1B). Reportable range of
171 ddPCR is from 10 copies/µl to 2500 copies/µl for both ORF1ab and N primes/probe
172 sets. In contrast, the dynamic range of RT-PCR is from 50 copies/µl to 10^5 copies/µl
173 for both ORF1ab and N primes/probe sets (Fig. 1C and 1D). To define the limit of
174 quantification of ddPCR, five low concentrations of plasmid control were analyzed
175 with 8 replicates. The lower limit of quantitation (LLoQ) of the optimized ddPCR is
176 1.003 copies/µl and 0.415 copies/µl for ORF1ab and N primers/probe sets,
177 respectively. The lower limit of detection (LLoD) of the optimized ddPCR is 0.109

178 copies/μl and 0.021 copies/μl for ORF1ab and N primers/probe sets, respectively (Fig.
179 2), which is at least 500 times lower than the RT-PCR detection kit used in current
180 clinical test. Therefore, the ddPCR is more sensitive for samples with low level
181 analyte.
182
183 **Detection of SARS-CoV-2 from patient specimens with ddPCR**
184 57 clinical pharyngeal swab samples (Fig. 3), which were judged to be negative by
185 both officially approved clinical RT-PCR detection and the commercial RT-PCR
186 detection kit for double check (generally referred to as RT-PCR), were tested with
187 ddPCR in double-blind. We did not know any information, results of clinical
188 diagnosis and status of enrolled patients during the tests. The follow-up investigation
189 revealed those information after ddPCR tests. Compared with the information and
190 clinical diagnosis, our results show that the overall accuracy of the optimized ddPCR
191 is 94.3 % and 64.2 % of supposed convalescents are still carrying SARS-CoV-2.
192 Details are as follows (Fig. 3) (Table 1 and 2):
193 Firstly, among 27 febrile suspected patients whose SARS-CoV-2 nucleic acid were
194 negative initially tested by RT-PCR, 25 out of 27 were detected with ddPCR as
195 positive and 2 out of 27 were negative. However, all 27 patients were diagnosed with
196 SARS-CoV-2 infection by chest CT as well as RT-PCR in subsequent follow-up
197 investigations, and all of them were hospitalized. As a result, 92.6 % of patients with
198 false negative nucleic acid test could be identified as positive by the optimized ddPCR
199 (Table 1).
200 Secondly, pharyngeal swabs of 8 febrile patients with negative results tested by
201 RT-PCR were also tested negative by ddPCR. In the follow-up investigation
202 COVID-19 was excluded based on the normal results of chest CT and RT-PCR (Table
203 1).
204 Thirdly, pharyngeal swabs collected from 8 febrile suspected patients in the clinic
205 recently with negative nucleic acid tests by RT-PCR, were detected positive by
206 ddPCR. However, chest CT of these 8 patients did not show any abnormalities upon
207 their first visit the clinic. According to official clinical guidelines, these 8 patients

were home quarantined and no further followed-up by us (Table 1).

Finally, pharyngeal swabs of 14 supposed convalescent were tested negative in two consecutive tests by RT-PCR (Table 2). However, using ddPCR, 9 out of 14 were positive with a positive rate of 64.2 %. Therefore, the current clinical practice that the convalescent continues to be quarantined for 2 weeks is reasonable and necessary.

In conclusion, compared with RT-PCR, ddPCR show superiority for clinical detection of SARS-CoV-2 to reduce the false negatives, which could be a powerful complement to the current standard RT-PCR.

Discussion

More and more nucleic acid detection kits have been developed for SARS-CoV-2 recently based on RT-PCR to meet the requirement of large-scale clinical molecular diagnosis. It has been reported that 6 kinds of RT-PCR detection kits were compared and analyzed for their detection performance. Results showed that there are differences in the detection ability of these kits for weakly positive samples, and the accuracy, sensitivity and reproducibility of some reagents are not ideal [17] In the meantime, many efforts have been focusing on developing better and complementary technology for clinical diagnosis of SARS-CoV-2, due to the limited sensitivity and precision of RT-PCR for viral quantitation. Different from RT-PCR that the data are measured from a single amplification curve and a Cq value, which is highly dependent on reaction efficiency, primer dimers and sample contaminants, ddPCR is measured at reaction end point which virtually eliminates these potential pitfalls. Results in this work proved that ddPCR is more sensitive (Fig. 1) and accurate for low viral load diagnosis (Fig. 2), which can greatly reduce the false negatives detection (Fig 3).

Based on two primers/probe sets targeting ORF1ab and N of SARS-CoV-2, results showed that N primers/probe set was more sensitive compared to that of ORF1ab. Among 42 samples that were judged as positive with ddPCR, 40 in 42 were detected as positive by N primers/probe set, and 12 in 42 were detected as positive by ORF1ab primers/probe set. This could be explained by the subgenomic RNA discontinuous

replication and transcription model of coronavirus. The genome RNA of SARS-CoV-2 encodes single copy of ORF1ab and N, respectively. In contrast, a nested set of around 10 subgenomic RNAs (sgRNAs), each of which encodes one copy of N, are synthesized by viral replication and transcription complex in a manner of discontinuous transcription .[18,19,20] Therefore, the copy numbers of N gene is significantly higher than that of ORF1ab gene in SARS-CoV-2 infected cells.

Although 2 patients, who were clinically confirmed by chest CT and RT-PCR subsequently, were reported as negative nucleic acid in pharyngeal swabs by our ddPCR, leading to 2 false negative reports by ddPCR in 35 cases (5.7 % missing rate), the overall accuracy of SARS-CoV-2 detection is significantly improved, which will benefit to the early diagnosis, intervention and treatment.

Notably, 64.2 % supposed convalescent patients, who are negative for pharyngeal swab nucleic acid tests twice by RT-PCR, are still carrying SARS-CoV-2 based on our work. Although there is no evidence that such COVID-19 convalescent carrying SARS-CoV-2 will be infectious to other healthy person, the risk still exists. Therefore, the current clinical practice that the convalescent continues to be quarantined for 2 weeks is reasonable and necessary. And we recommend that ddPCR could be a complement to the current standard RT-PCR to re-confirm the convalescent, which will benefit to reduce the risk of the SARS-CoV-2 epidemic and social panic.

Author Contributions

YC, KL conceptualized the study design. TS, WH, LD, TC, YX, and GC recruited the patients, collected specimens, collected demographic, clinical data; XL, MG, QZ, XW, YY, MS, DG and ZH did the laboratory tests. JF, YL and QZ plotted the figures; XL, MG, JF and YC analyzed the data; ZH, XK, YL, YlL and YC interpreted the results; JF wrote the initial drafts of the manuscript; YC and KL revised the manuscript and FL and KX commented on it. All authors read and approved the final report.

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Declaration of interests

No authors have received research funding from the company whose commercial products were used in this work. All authors report no competing interests. All authors have completed the Unified Competing Interest form.

Reference

1. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020; https://doi.org/10.1038/s41586-020-2008-3
2. Chen L, Liu W, Zhang Q, et al. RNA based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak. Emerg Microbes Infect 2020;9(1):313–9.
3. World Health Organization. Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. [Internet]. 2020; Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-g

uidance/laboratory-guidance

4. General Office of the National Health and Health Commission O of the SA of TCM. Diagnosis and treatment of pneumonitis with a new type of coronavirus infection (trial version 5) [Internet]. 2020;Available from: http://bgs.satcm.gov.cn/zhengcewenjian/2020-02-06/12848.html

5. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72□314 Cases From the Chinese Center for Disease Control and Prevention. JAMA [Internet] 2020;2019:25–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32091533

6. Vogelstein B, Kinzler KW. Digital PCR. Proc Natl Acad Sci U S A 1999;96(16):9236–41.

7. Pohl G, Shih I-M. Principle and applications of digital PCR. Expert Rev Mol Diagn 2004;4(1):41–7.

8. Sanders R, Mason DJ, Foy CA, Huggett JF. Evaluation of Digital PCR for Absolute RNA Quantification. PLoS One 2013;8(9):e75296.

9. White RA, Blainey PC, Fan HC, Quake SR. Digital PCR provides sensitive and absolute calibration for high throughput sequencing. BMC Genomics 2009;10(1):110–6.

10. Hindson BJ, Ness KD, Masquelier DA, et al. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal Chem 2011;83(22):8604–10.

11. Hindson CM, Chevillet JR, Briggs HA, et al. Absolute quantification by droplet digital PCR versus analog real-time PCR. Nat Methods 2013;10(10):1003–5.

12. Brunetto GS, Massoud R, Leibovitch EC, et al. Digital droplet PCR (ddPCR) for the precise quantification of human T-lymphotropic virus 1 proviral loads in peripheral blood and cerebrospinal fluid of HAM/TSP patients and identification of viral mutations. J Neurovirol 2014;20(4):341–51.

13. Caviglia GP, Abate ML, Tandoi F, et al. Quantitation of HBV cccDNA in

anti-HBc-positive liver donors by droplet digital PCR: A new tool to detect occult infection. J Hepatol 2018;69(2):301–7. Available from: https://doi.org/10.1016/j.jhep.2018.03.021

14. Postel M, Roosen A, Laurent-Puig P, Taly V, Wang-Renault S-F. Droplet-based digital PCR and next generation sequencing for monitoring circulating tumor DNA: a cancer diagnostic perspective. Expert Rev Mol Diagn 2018;18(1):7–17.

15. Miyaoka Y, Mayerl SJ, Chan AH, Conklin BR. Detection and Quantification of HDR and NHEJ Induced by Genome Editing at Endogenous Gene Loci Using Droplet Digital PCR [Internet]. In: Karlin-Neumann G, Bizouarn F, editors. Digital PCR: Methods and Protocols. New York, NY: Springer New York; 2018. p. 349–62.Available from: https://doi.org/10.1007/978-1-4939-7778-9_20

16. National Institute For viral Disease Control and prevention of PRC. Specific primers and probes for detection 2019 novel coronavirus [Internet]. 2020; Available from: http://www.chinaivdc.cn/kyjz/202001/t20200121_211337.html

17. Guo Y., Wang K., Zhang Y., Zhang W., Wang L. LP. Comparison and analysis of the detection performance of six new coronavirus nucleic acid detection reagents. Chongqing Med 2020;14(0):1671–8348.

18. Thiel V, Ivanov KA, Putics Á, et al. Mechanisms and enzymes involved in SARS coronavirus genome expression. J Gen Virol 2003;84(9):2305–15.

19. Hussain S, Pan J, Chen Y, et al. Identification of Novel Subgenomic RNAs and Noncanonical Transcription Initiation Signals of Severe Acute Respiratory Syndrome Coronavirus. J Virol 2005;79(9):5288–95.

20. Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. J Med Virol 2020;(January):1–6.

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Figure legends

Figure 1. Plot of results from a linearity experiment to determine reportable range of ddPCR and RT-PCR targeting for ORF1ab and N of SARS-CoV-2. (A and B) Expected values (converted to log10) were plotted on the X axis versus measured values (converted to log10) on the Y axis using Graph Pad Prism for ddPCR targeting ORF1ab and N. (C and D) Expected values (converted to log10) were plotted on the X axis versus measured Ct values on the Y axis using Graph Pad Prism for RT-PCR targeting ORF1ab and N. Data are representative of three independent experiments with 3 replicates for each concentration.

Figure 2. Probit analysis sigmoid curve reporting the lower limit of quantitation (LLoQ) and the lower limit of detection (LLoD) of ddPCR. Replicate reactions of SARS-CoV-2 (A) ORF1ab and (B) N were done at concentrations around the detection end point determined in preliminary dilution experiments. The X axis shows expected concentration (copies/µl). The Y axis shows fraction of positive results in all parallel reactions performed. The inner line is a probit curve (dose-response rule). The outer lines are 95 % CI. Data are representative of three independent experiments with 8 replicates for each concentration.

Figure 3. Information diagram of detection results with ddPCR and subsequent clinical diagnosis for both convalescent and febrile suspected patients.

Tables

Table 1. Detection results of ddPCR for febrile and suspected patients of COVID-19.

Patient Number	Patient status	Result of official nucleic acid test by RT-PCR	Result of nucleic acid test by RT-PCR in lab	Result of ddPCR (copies/μl)		Judgment result of ddPCR	Result of chest CT	Disposition of Hospital
				ORF1ab	N			
P1	Fever, suspected	Negative	Negative	0	0.1	Positive	Viral pneumonia	Hospitalized
P2	Fever, suspected	Negative	Negative	0	0.1	Positive	Viral pneumonia	Hospitalized
P3	Fever, suspected	Negative	Negative	0	0.1	Positive	Viral pneumonia	Hospitalized
P4	Fever, suspected	Negative	Negative	0	0.09	Positive	Viral pneumonia	Hospitalized
P5	Fever, suspected	Negative	Negative	0	0.07	Positive	Viral pneumonia	Hospitalized
P6	Fever, suspected	Negative	Negative	0	0.18	Positive	Viral pneumonia	Hospitalized
P7	Fever, suspected	Negative	Negative	0.15	0.68	Positive	Viral pneumonia	Hospitalized
P8	Fever, suspected	Negative	Negative	0.08	0.66	Positive	Viral pneumonia	Hospitalized

P9	Fever, suspected	Negative	Negative	0	0.08	Positive	Viral pneumonia	Hospitalized
P10	Fever, suspected	Negative	Negative	0	0.18	Positive	Viral pneumonia	Hospitalized
P11	Fever, suspected	Negative	Negative	0	0.23	Positive	Viral pneumonia	Hospitalized
P12	Fever, suspected	Negative	Negative	0.1	0.19	Positive	Viral pneumonia	Hospitalized
P13	Fever, suspected	Negative	Negative	0	0.18	Positive	Viral pneumonia	Hospitalized
P14	Fever, suspected	Negative	Negative	0	0.09	Positive	Viral pneumonia	Hospitalized
P15	Fever, suspected	Negative	Negative	0	0.37	Positive	Viral pneumonia	Hospitalized
P16	Fever, suspected	Negative	Negative	0	0.09	Positive	Viral pneumonia	Hospitalized
P17	Fever, suspected	Negative	Negative	0	0.16	Positive	Viral pneumonia	Hospitalized
P18	Fever, suspected	Negative	Negative	0.19	0.09	Positive	Viral pneumonia	Hospitalized
P19	Fever, suspected	Negative	Negative	0.1	0	Positive	Viral pneumonia	Hospitalized
P20	Fever, suspected	Negative	Negative	0	0.1	Positive	Viral pneumonia	Hospitalized
P21	Fever, suspected	Negative	Negative	0	0.33	Positive	Viral pneumonia	Hospitalized

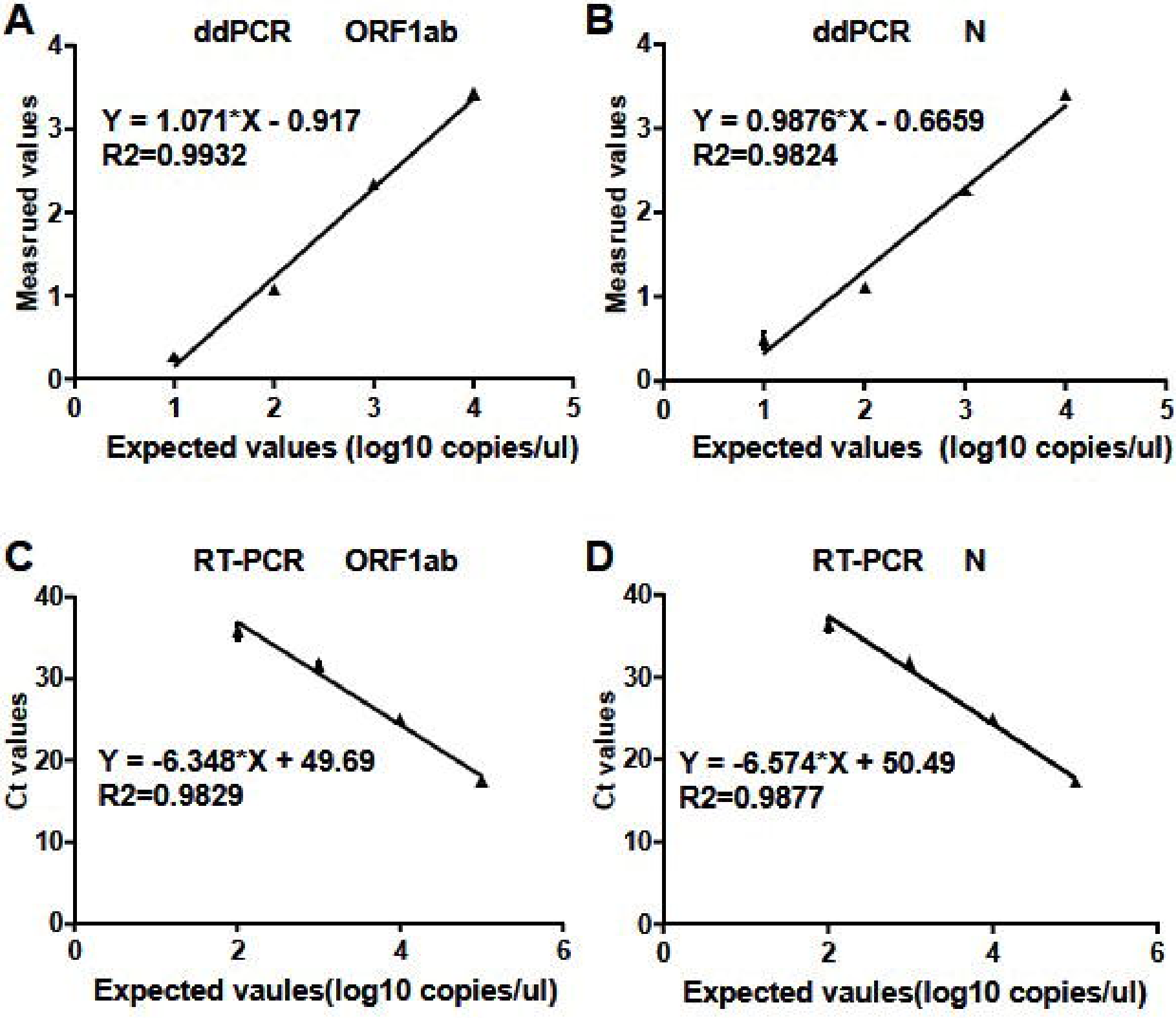
P22	Fever, suspected	Negative	Negative	0.22	0.71	Positive	Viral pneumonia	Hospitalized
P23	Fever, suspected	Negative	Negative	0	0.16	Positive	Viral pneumonia	Hospitalized
P24	Fever, suspected	Negative	Negative	0	0.09	Positive	Viral pneumonia	Hospitalized
P25	Fever, suspected	Negative	Negative	0	0.16	Positive	Viral pneumonia	Hospitalized
P26	Fever, suspected	Negative	Negative	0	0	Negative	Viral pneumonia	Hospitalized
P27	Fever, suspected	Negative	Negative	0	0	Negative	Viral pneumonia	Hospitalized
P28	Fever, suspected	Negative	Negative	0	0.17	Positive	Normal	Home Quarantine
P29	Fever, suspected	Negative	Negative	0	0.06	Positive	Normal	Home Quarantine
P30	Fever, suspected	Negative	Negative	0	0.06	Positive	Normal	Home Quarantine
P31	Fever, suspected	Negative	Negative	0.08	0.2	Positive	Normal	Home Quarantine
P32	Fever, suspected	Negative	Negative	0	0.19	Positive	Normal	Home Quarantine
P33	Fever, suspected	Negative	Negative	0	0.279	Positive	Normal	Home Quarantine

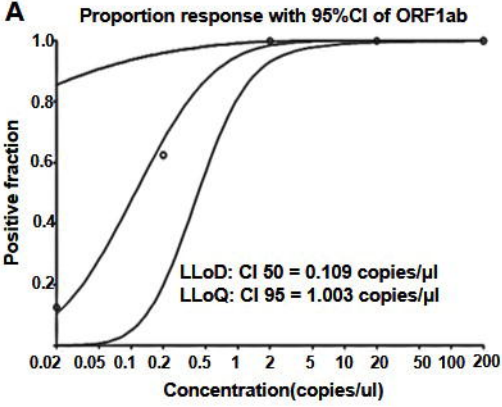
P34	Fever, suspected	Negative	Negative	0.15	0.8	Positive	Normal	Home Quarantine
P35	Fever, suspected	Negative	Negative	0	0.1	Positive	Normal	Home Quarantine
P36	Fever, suspected	Negative	Negative	0	0	Negative	Normal	Excluded
P37	Fever, suspected	Negative	Negative	0	0	Negative	Normal	Excluded
P38	Fever, suspected	Negative	Negative	0	0	Negative	Normal	Excluded
P39	Fever, suspected	Negative	Negative	0	0	Negative	Normal	Excluded
P40	Fever, suspected	Negative	Negative	0	0	Negative	Normal	Excluded
P41	Fever, suspected	Negative	Negative	0	0	Negative	Normal	Excluded
P42	Fever, suspected	Negative	Negative	0	0	Negative	Normal	Excluded
P43	Fever, suspected	Negative	Negative	0	0	Negative	Normal	Excluded

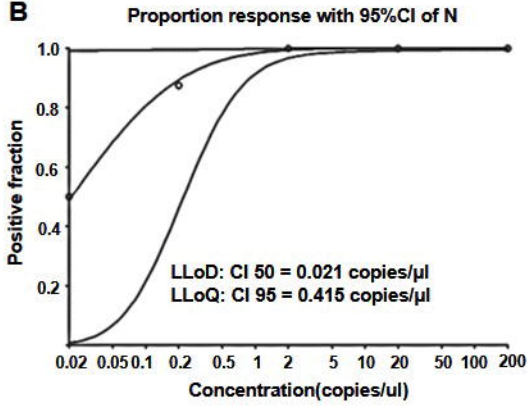
Table 2. Detection results of ddPCR for supposed convalescent patients who is about to be discharged after treatment.

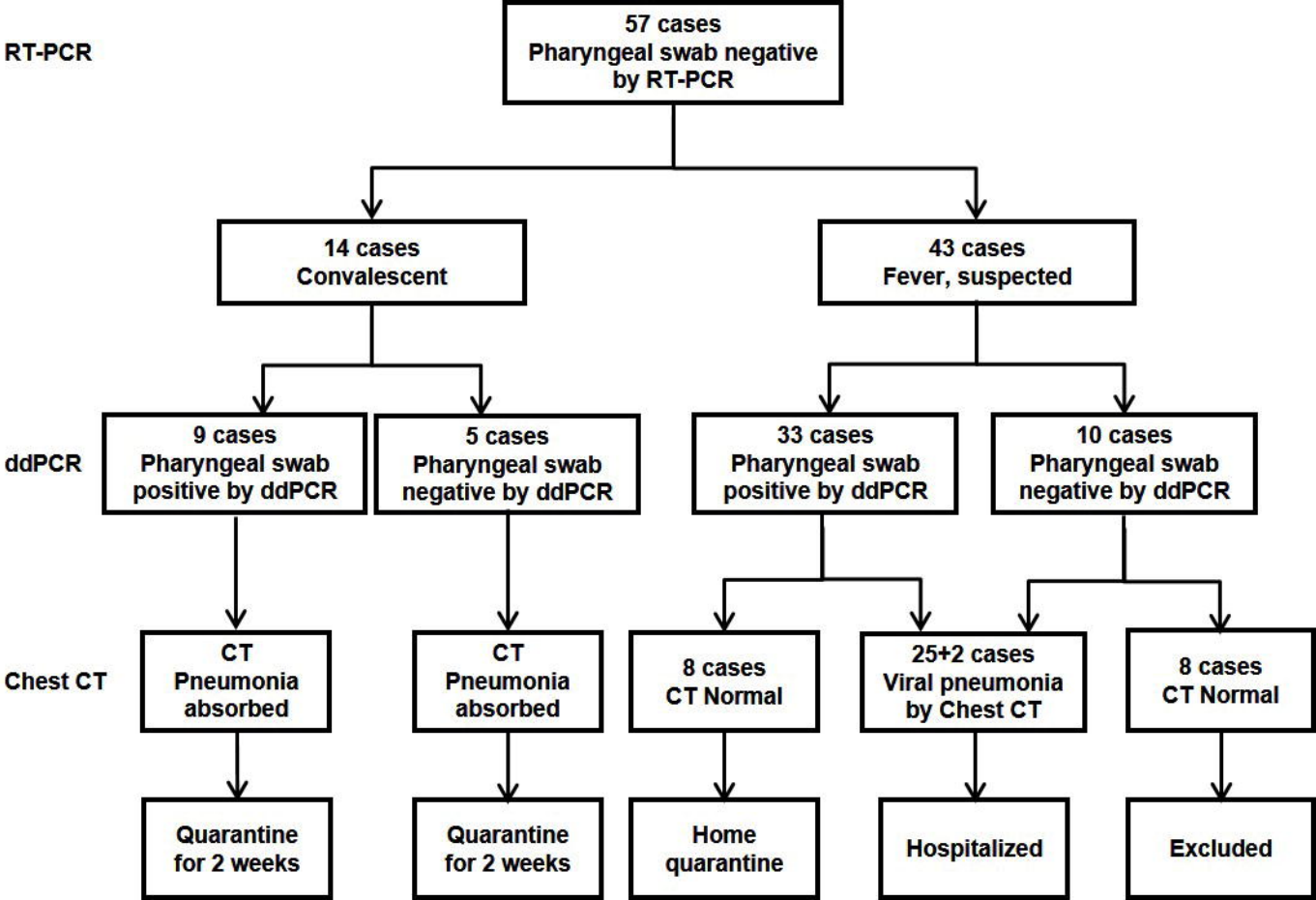
Patient Number	Patient status	Result of official nucleic acid test by real time PCR (Positive/Negative)	Result of nucleic acid test by RT-PCR in our lab	Result of ddPCR (copies/μl)		Judgment result of ddPCR (Positive/Negative)
				ORF1ab	N	
P44	Supposed convalescent	Negative	Negative	0	0.12	Positive
P45	Supposed convalescent	Negative	Negative	0	0.11	Positive
P46	Supposed convalescent	Negative	Negative	0.57	0.6	Positive
P47	Supposed convalescent	Negative	Negative	0	0.45	Positive
P48	Supposed convalescent	Negative	Negative	0	0.8	Positive
P49	Supposed convalescent	Negative	Negative	0.09	0	Positive
P50	Supposed convalescent	Negative	Negative	0	0.11	Positive
P51	Supposed convalescent	Negative	Negative	0.19	5.3	Positive
P52	Supposed convalescent	Negative	Negative	0.07	0.07	Positive

P53	Supposed convalescent	Negative	Negative	0	0	Negative
P54	Supposed convalescent	Negative	Negative	0	0	Negative
P55	Supposed convalescent	Negative	Negative	0	0	Negative
P56	Supposed convalescent	Negative	Negative	0	0	Negative
P57	Supposed convalescent	Negative	Negative	0	0	Negative


A
ddPCR ORF1ab
Y = 1.071*X - 0.917
R2=0.9932
Measrued values
Expected values (log10 copies/ul)
B
ddPCR N
Y = 0.9876*X - 0.6659
R2=0.9824
Measrued values
Expected values (log10 copies/ul)
C
RT-PCR ORF1ab
Y = -6.348*X + 49.69
R2=0.9829
Ct values
Expected vaules(log10 copies/ul)
D
RT-PCR N
Y = -6.574*X + 50.49
R2=0.9877
Ct values
Expected vaules(log10 copies/ul)


A
Proportion response with 95%CI of ORF1ab
Positive fraction
1.0
0.8
0.6
0.4
0.2
LLoD: CI 50 = 0.109 copies/μl
LLoQ: CI 95 = 1.003 copies/μl
0.02 0.05 0.1 0.2 0.5 1 2 5 10 20 50 100 200
Concentration(copies/ul)


B
Proportion response with 95%CI of N
Positive fraction
1.0
0.8
0.6
0.4
0.2
LLoD: CI 50 = 0.021 copies/μl
LLoQ: CI 95 = 0.415 copies/μl
0.02 0.05 0.1 0.2 0.5 1 2 5 10 20 50 100 200
Concentration(copies/ul)


RT-PCR
57 cases
Pharyngeal swab negative by RT-PCR
14 cases
Convalescent
43 cases
Fever, suspected
ddPCR
9 cases
Pharyngeal swab positive by ddPCR
5 cases
Pharyngeal swab negative by ddPCR
33 cases
Pharyngeal swab positive by ddPCR
10 cases
Pharyngeal swab negative by ddPCR
Chest CT
CT
Pneumonia absorbed
CT
Pneumonia absorbed
8 cases
CT Normal
25+2 cases
Viral pneumonia by Chest CT
8 cases
CT Normal
Quarantine for 2 weeks
Quarantine for 2 weeks
Home quarantine
Hospitalized
Excluded