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

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32 **Abstract**

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

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

44 **Results:** The lower limit of detection of the optimized ddPCR is at least 500 times
45 lower than that of RT-PCR. The overall accuracy of ddPCR for clinical detection is
46 94.3 %. 33 out of 35 negative pharyngeal swab samples checked by RT-PCR were
47 correctly judged by ddPCR based on the follow-up investigation. In addition, 9 out of
48 14 (64.2 %) supposed convalescents with negative nucleic acid test twice by RT-PCR
49 were positive by ddPCR detection.

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

55

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

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58 **Introduction**

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

81

82 Digital PCR is based on the principles of limited dilution, end-point PCR, and Poisson
83 statistics, with absolute quantification as its heart.[6] It has broader dynamic range
84 without external interference and robustness to variations in PCR efficiency. [7–9] In
85 2011, Hindson developed the droplet digital PCR (ddPCR) technology based on
86 traditional digital PCR.[10] The reaction mixture can be divided into tens of thousands
87 of nanodroplets during the process. These vast and highly consistent oil droplets

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88 substantially improve the detection dynamic range and accuracy of digital PCR in a
89 low-cost and practical format.[11] In recent years, this technology has been widely used,
90 such as analysis of absolute viral load from clinical samples, analysis of gene copy
91 number variation, rare allele detection, gene expression, microRNA analysis and
92 genome edit detection *et al*.[12,13,14,15] Here, taking the advantages of ddPCR, we
93 optimized the preparation of pharyngeal swab samples, and develop a workflow of
94 ddPCR to detect SARS-CoV-2 using Chinese CDC approved primer and probe sets.
95 Based on the results of this optimized ddPCR system, we showed that the overall
96 accuracy of the ddPCR for clinical pathogen detection is 94.3 %, and 64.2 % of
97 supposed convalescents with two consecutive negative nucleic acid tests by RT-PCR
98 still carry SARS-CoV-2.
99
100 **Materials and methods**
101
102 **Ethics statement**
103 This study was approved by the Ethics Committee of the Renmin Hospital and
104 Zhongnan Hospital of Wuhan University. The analysis was performed on existing
105 samples collected during standard diagnostic tests, posing no extra burden to patients,
106 as described previously.[2]
107
108 **Specimen collection and RNA extraction**
109 Pharyngeal swab samples were obtained from clinical suspected patients with fever or
110 rehabilitation quasi-discharged patients of COVID-19 at Renmin Hospital and
111 Zhongnan Hospital of Wuhan University according to the interim guidance of WHO.
112 Pharyngeal swabs were soaked in 500 µl PBS and vortexed with diameter of 3 mm
113 beads (Novastar, China) for 15 seconds immediately. Total RNA was extracted from
114 the supernatant using QIAamp viral RNA mini kit (Qiagen) following manufacturer's
115 instruction. First strand cDNA was synthesized using PrimeScript RT Master Mix
116 (TakaRa) with random primer and oligo dT primer.
117

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118 **Primers and probes**

119 The primers and probes targeted the ORF1ab and N of SARS-CoV-2 according to
120 Chinese CDC. Target 1 (ORF1ab), forward: 5'-CCCTGTGGGTTTTACACTTAA-3',
121 reverse: 5'-ACGATTGTGCATCAGCTGA-3', probe:
122 5'-FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3';
123 Target 2 (N), forward: 5'-GGGGAACTTCTCCTGCTAGAAT-3',
124 reverse: 5'-CAGACATTTTGCTCTCAAGCTG-3',
125 probe: 5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3'.[16]

126

127 **Droplet Digital PCR workflow**

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

140

141 **RT-PCR**

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

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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

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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

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208 were home quarantined and no further followed-up by us (Table 1).
209 Finally, pharyngeal swabs of 14 supposed convalescent were tested negative in two
210 consecutive tests by RT-PCR (Table 2). However, using ddPCR, 9 out of 14 were
211 positive with a positive rate of 64.2 %. Therefore, the current clinical practice that the
212 convalescent continues to be quarantined for 2 weeks is reasonable and necessary.
213 In conclusion, compared with RT-PCR, ddPCR show superiority for clinical detection
214 of SARS-CoV-2 to reduce the false negatives, which could be a powerful complement
215 to the current standard RT-PCR.
216

217 **Discussion**

218 More and more nucleic acid detection kits have been developed for SARS-CoV-2
219 recently based on RT-PCR to meet the requirement of large-scale clinical molecular
220 diagnosis. It has been reported that 6 kinds of RT-PCR detection kits were compared
221 and analyzed for their detection performance. Results showed that there are
222 differences in the detection ability of these kits for weakly positive samples, and the
223 accuracy, sensitivity and reproducibility of some reagents are not ideal [17] In the
224 meantime, many efforts have been focusing on developing better and complementary
225 technology for clinical diagnosis of SARS-CoV-2, due to the limited sensitivity and
226 precision of RT-PCR for viral quantitation. Different from RT-PCR that the data are
227 measured from a single amplification curve and a Cq value, which is highly
228 dependent on reaction efficiency, primer dimers and sample contaminants, ddPCR is
229 measured at reaction end point which virtually eliminates these potential pitfalls.
230 Results in this work proved that ddPCR is more sensitive (Fig. 1) and accurate for low
231 viral load diagnosis (Fig. 2), which can greatly reduce the false negatives detection
232 (Fig 3).
233 Based on two primers/probe sets targeting ORF1ab and N of SARS-CoV-2, results
234 showed that N primers/probe set was more sensitive compared to that of ORF1ab.
235 Among 42 samples that were judged as positive with ddPCR, 40 in 42 were detected
236 as positive by N primers/probe set, and 12 in 42 were detected as positive by ORF1ab
237 primers/probe set. This could be explained by the subgenomic RNA discontinuous

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238 replication and transcription model of coronavirus. The genome RNA of
239 SARS-CoV-2 encodes single copy of ORF1ab and N, respectively. In contrast, a
240 nested set of around 10 subgenomic RNAs (sgRNAs), each of which encodes one
241 copy of N, are synthesized by viral replication and transcription complex in a manner
242 of discontinuous transcription .[18,19,20] Therefore, the copy numbers of N gene is
243 significantly higher than that of ORF1ab gene in SARS-CoV-2 infected cells.
244 Although 2 patients, who were clinically confirmed by chest CT and RT-PCR
245 subsequently, were reported as negative nucleic acid in pharyngeal swabs by our
246 ddPCR, leading to 2 false negative reports by ddPCR in 35 cases (5.7 % missing rate),
247 the overall accuracy of SARS-CoV-2 detection is significantly improved, which will
248 benefit to the early diagnosis, intervention and treatment.
249 Notably, 64.2 % supposed convalescent patients, who are negative for pharyngeal
250 swab nucleic acid tests twice by RT-PCR, are still carrying SARS-CoV-2 based on our
251 work. Although there is no evidence that such COVID-19 convalescent carrying
252 SARS-CoV-2 will be infectious to other healthy person, the risk still exists. Therefore,
253 the current clinical practice that the convalescent continues to be quarantined for 2
254 weeks is reasonable and necessary. And we recommend that ddPCR could be a
255 complement to the current standard RT-PCR to re-confirm the convalescent, which
256 will benefit to reduce the risk of the SARS-CoV-2 epidemic and social panic.
257

258 **Author Contributions**

259 YC, KL conceptualized the study design. TS, WH, LD, TC, YX, and GC recruited the
260 patients, collected specimens, collected demographic, clinical data; XL, MG, QZ, XW,
261 YY, MS, DG and ZH did the laboratory tests. JF, YL and QZ plotted the figures; XL,
262 MG, JF and YC analyzed the data; ZH, XK, YL, YlL and YC interpreted the
263 results; JF wrote the initial drafts of the manuscript; YC and KL revised the
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266

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283 **Declaration of interests**

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287

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363 **Figure legends**

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

372

373 **Figure 2.** Probit analysis sigmoid curve reporting the lower limit of quantitation
374 (LLoQ) and the lower limit of detection (LLoD) of ddPCR. Replicate reactions of
375 SARS-CoV-2 (A) ORF1ab and (B) N were done at concentrations around the
376 detection end point determined in preliminary dilution experiments. The X axis shows
377 expected concentration (copies/μl). The Y axis shows fraction of positive results in all
378 parallel reactions performed. The inner line is a probit curve (dose-response rule). The
379 outer lines are 95 % CI. Data are representative of three independent experiments with
380 8 replicates for each concentration.

381

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

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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

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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

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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.

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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

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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

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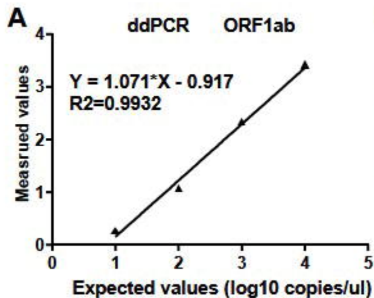
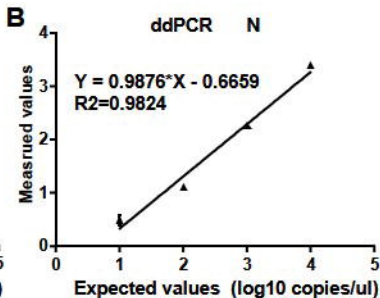
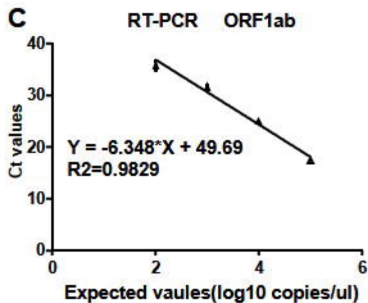
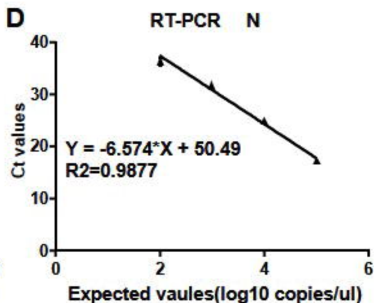

A
ddPCR ORF1ab
Y = 1.071*X - 0.917
R2=0.9932
Measrued values
Expected values (log10 copies/ul)


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

1.0
0.8
0.6
0.4
0.2

Positive fraction

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

1.0
0.8
0.6
0.4
0.2

Positive fraction

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