

Supplementary Information

Insufficient sensitivity of RNA dependent RNA polymerase Gene of SARS-CoV-2 viral genome as Confirmatory Test using Korean COVID-19 cases

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Methods

Case Report

Korean COVID-19 Case #3 and # 17 are presented at Reference #5

Korean COVID-19 Case # 22: A 46-year-old male who was positive for SARS-CoV-2 RT-PCR was admitted. He is the brother of the 16th confirmed COVID-19 patient in South Korea, who was diagnosed positive after returning from Thailand. He ate with his family on lunar New Year's Day on January 25, 2020, including the 16th confirmed COVID-19 patient. He was self-isolated shortly after the 16th COVID-19 patient was confirmed and was underwent a SARS-CoV-2 RT-PCR test. He was confirmed positive by the SARS-CoV-2 RT-PCR test and was admitted to the Chosun university hospital at February 06, 2020.

He was asymptomatic at the time of admission and had no unusual findings on physical examination. The vital sign was stable (BP 130/80mmHg, HR 78beats/min, RR 20/min, BT 36.2 °C). Laboratory investigations revealed a white blood cell count of $4.70 \times 10^3/\mu\text{L}$, hemoglobin level of 14.4 g/dL, and platelet count of $2.70 \times 10^3/\mu\text{L}$ on routine complete blood count. Serum biochemistry revealed the following: blood urea nitrogen, 10.0 mg/dL; creatinine, 0.8 mg/dL. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were 20.0 U/L and 31 U/L, respectively. In his first SARS-CoV-2 RT-PCR test sent to KCDC, the Ct values in the upper respiratory tract specimens (Nasopharynx) were 35.15 (RdRP) and 33.45 (E), and the Ct values in the following lower respiratory tract specimens (Sputum) were 30.51 (RdRP) and 28.47 (E). It was undetectable in urine.

Chest X-ray was normal at admission, but high resolution computed tomography revealed ground glass opacity (GGO) findings in left upper lung (LUL) field. After confirming pneumonia, he began taking lopinavir / ritonavir (Kaletra®). Although he complained of nonproductive coughing from Day 2 of hospitalization and Diarrhea from Day 3 of hospitalization, these symptoms were self-limiting after several days. Pneumonia of the LUL area was prominently identified on the follow-up chest X-ray, and the GGO at the LUL worsened on the HRCT confirmed on the 6th day of hospitalization. However, the patient's symptoms were minimal. 48 hours later symptoms disappeared, SARS-CoV-2 RT-PCR was performed at 24 hours intervals on upper and lower respiratory tract samples. As a result, no virus was detected in the upper and lower respiratory tract samples following up on the 6th, 8th, and 9th days of hospitalization, in addition, all urine and stool samples performed at that time were also negative. Based on these results, the patient was discharged to a stable state.

RT-PCR CONFIRMATORY TESTS

Primer and probe sequences in this study were based upon WHO guidance

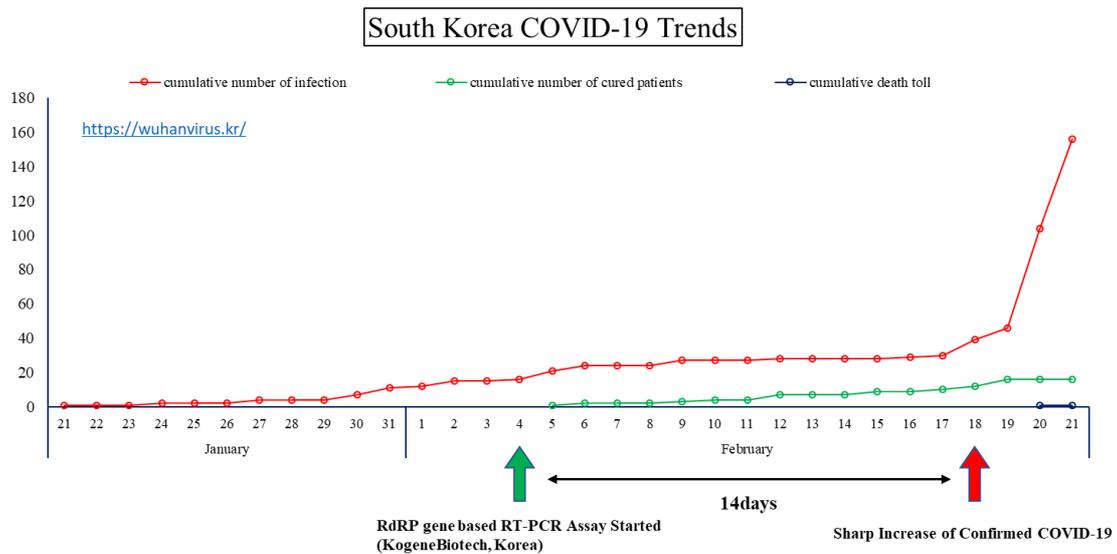
(https://www.who.int/docs/default-source/coronaviruse/uscdcr-pcr-panel-primer-probes.pdf?sfvrsn=fa29cb4b_2), as well as US CDC panel primer and probes updated in 28 Jan, 2020 (<https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-for-detection-instructions.pdf>).

All oligonucleotides were synthesized and provided by IDT. A 25 µl reaction was set up containing 5 µl of RNA, 12.5 µl of 2 X reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen; containing 0.4 mM of each deoxyribonucleotide triphosphates (dNTP) and 3.2 mM magnesium sulfate), 1 µl of reverse transcriptase/Taq mixture from the kit. Thermal cycling was performed at 50°C for 30 min for reverse transcription, followed by 95°C for 2min and then 40 cycles of 95°C for 15 s, 55°C for 1min. All the information including Primer and Probe Mixture are included the kit insert (PCLMD™ nCoV one step RT-PCR Kit from PCL Inc, Korea, Cat # PCLMD2019001-50).

Supplementary Table 1. Figure 1A Data
(Value are Ct value, and over 35 is considered as Negative)

		Korean COVID-19 Case #22			PC RNA	NC
		Test #1	Test #2	Test #3		
Internal control	RNase P	35.97	32.13	29.56	34.73	ND
Screening_SARS-CoV-2	E gene	30.07	30.06	30.07	26.69	ND
Confirmatory_SARS-CoV-2	RdRp gene (WHO, KCDC)	30.12	30.06	29.82	31.15	39.84
	N gene (CDC)	29.90	29.82	29.94	16.30	ND
	N1+N3 (PCL Inc)	27.66	27.69	27.73	28.64	ND

Supplementary Figure 1. Trends in COVID-19 case in Korea



On Feb 4th, Korean CDC advised the hospitals to use the diagnostic method based on RdRP gene (KogeneBiotech, Korea). Surprisingly, after using the diagnostic method to RdRP gene-based RT-PCR confirmatory test, Korean COVID-19 confirmed cases did not increase much. However, from Feb 17, after 14 days of using RdRP based assay, COVID-19 cases started to increase sharply. We suspect that early, low titer asymptomatic COVID-19 patients have not been detected using RdRP gene-based RT-PCR test, thus these false negative patients might have spread the COVID-19 after latent or asymptomatic period (from Feb 4 to Feb 18, 14 days).