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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Since mid-December of 2019, coronavirus disease 2019 (COVID-19) has been spreading from Wuhan, China. As of February 21, total 75,773 confirmed cases worldwide have spread to more than two dozen countries.

The first confirmed COVID-19 patients in South Korea are those who came from or visited China. Lately, secondary and tertiary spreads have occurred, and the speed of transmission is accelerating. South Korea has 104 confirmed cases of the COVID-19 and first death case broke. Investigations are underway worldwide to better understand transmission dynamics.

Transmission of COVID-19 can occur early in the course of infection since SARS-CoV-2 viral loads in asymptomatic patients are similar to that in the symptomatic patients.[1] This indicates asymptomatic or minimally symptomatic patients with low levels of detectable viral RNA can transmit the COVID-19.[2] Therefore, more sensitive diagnostic methods are needed to detect early phase of the infection to prevent secondary or tertiary spreads.

Currently, the detection method for SARS-CoV-2 is based on viral RNA detection using Reverse Transcribed Real-Time PCR (RT-PCR). WHO initially distributed the guideline for confirming COVID-19, based on RNA dependent RNA polymerase (RdRP) viral RNA gene detecting RT-PCR method.[3] Accordingly, Korea Centers for Disease Control & Prevention (KCDC) has used confirmatory RT-PCR based on RdRP gene. On the other hand, US CDC recommended to use SARS-CoV-2 nucleocapsid protein (N) genes instead of RdRP gene as a confirmatory test.[4]

Here, we compare the RT-PCR confirmatory test results using two different SARS-CoV-2 viral RNAs from two Korean COVID-19 confirmed cases. Figure 1A shows that RT-PCR targeting the N gene is more sensitive than targeting the RdRP gene by 7- 43 fold, based on Ct value difference, in SARS-CoV-2 whole-genome RNA of Korean COVID-19 cases. Moreover, RdRP gene-based confirmatory test could not detect the virus from case # 17 which led to false negatives in the early stage.

According to this information, we further formulated the N genes (N1+N3) with highest sensitivity for RT-PCR confirmatory test and follow sensitivity of one specimen from Korean COVID case #3.[5] In the case of #3, during administration of anti-HIV agent,

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lopinavir/ritonavir, RdRP gene confirmatory test (KogeneBiotech, Korea) was negative. However, confirmatory test using the N gene (PCL Inc.) turned out to be positive in Day 10 (Fig 1B). This implicates that low viral titer cannot be detected from RdRP gene confirmatory test currently used in Korea according to WHO guideline.

Protein-coding RNAs in cells differ in their absolute quantity and stability. In this regard, in the RT-PCR-based diagnostic method, it is desirable to detect the viral target RNA with high abundance and stability to maximize detection sensitivity and accuracy. Our results demonstrate that in the case of SARS-CoV-2, RT-PCR targeting N gene show lower Ct value and more reproducible detection than RdRP, which might reflect the different RNA abundance and/or stability. Further study is needed to confirm this hypothesis.

In conclusion, to prevent the further spread of COVID-19 by the early-stage patient, WHO should consider recommending the use of N gene rather than RdRP gene for confirmatory test.

Figure 1. RT-PCR assay targeting viral N gene can increase the sensitivity of detection of COVID-19

A. RT-PCR assay targeting the RdRP gene (WHO, KCDC, KogeneBiotech) is less sensitive than the one targeting N genes (CDC, PCL Inc). In the case of Whole Genome (SARS-CoV-2 RNA), N gene can detect the virus with sensitivity increased by ~43 fold (Ct Value difference 14) compared with the RdRP gene. In the case of Korean COVID-19 Case #17, N gene can detect the virus with ~10 fold increased sensitivity (Ct Value difference 3.4) compared with the RdRP gene. In the case of Korean COVID-19 Case #22, the sensitivity difference is ~7 fold (Ct Value difference 2.3).

B. RT-PCR assay targeting a mixture of N genes(N1+N3) show the highest sensitivity (PCLMD™ nCoV one step RT-PCR Kit from PCL Inc, Seoul, Korea) and confirms Korean COVID-19 case #3, who was diagnosed as false-negative by the RdRP test. Day 10 after the symptom emerged, viral RNA from COVID-19 Case #3 patient can be detected by N gene-based RT-PCR, but not by RdRP gene-based RT-PCR.

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Ethics Statement

Myongji Hospital Institutional Review Board (IRB) approved this study (No. IRB 2020-01-027) and written informed consent was given by the patient.

Author Disclosure Statement

The authors have nothing to disclose.