Effect of virus variants on COVID-19 diagnosis in the Republic of Korea

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Since the announcement of the outbreak of pneumonia of unknown cause in China on December 31, 2019, the global pandemic of coronavirus disease (COVID-19) has continued. The causative pathogen of COVID-19 was first identified as SARS-CoV-2 on 6, January 2021. The entire genome of the virus was analyzed and first registered with the Global Initiative on Sharing All Influenza Data (GISAID) [1]. SARS-CoV-2 is a positive single-stranded RNA (+ssRNA) virus that can mutate at a relatively higher frequency than DNA viruses in the replication process due to its genetic characteristics. In fact, SARS-CoV-2 has been reported to have various variants since its first identification, and World Health Organization (WHO) has classified them as variants of concern (VOC) and variants of interests (VOI) for public health response against SARS-CoV-2 variants [1]. VOC has mutated the gene site encoding spike protein (S) and classified as alpha (α), beta (β), gamma (γ), and delta (δ). Variant virus basically increases the transmissibility and severity of pathogens and reduces vaccine effectiveness [2]. The main mutant locations for each variant virus can be specified as alpha: N501Y, beta: N501Y, E484K, K417N, gamma: N501Y, E484K, delta: T478K, L452R, and P681R (Figure 1), mainly occurring in the S1 subunit of the gene encoding spike protein (S) [2-4].

In the Republic of Korea (ROK), the epidemic status of these VOC is continuously monitored. In July 2021, the total variants viruses detection rate was 58.7%, and the detection rate for each variant was as follows: alpha 8.9%, beta 0.1%, gamma 0.1%, and delta 49.7%. More than 50% of SARS-CoV-2 popular in the ROK was occupied by VOC.

As the occurrence of variant viruses increases, it is necessary to check whether the domestic COVID-19 diagnostic test method has any difficulties detecting variant viruses. Therefore, the purpose of this study was to determine whether the COVID-19 diagnostic



Figure 1. SARS-CoV-2 target gene site and major mutant virus mutation site

test method based on the detection (amplification) of the gene of SARS-CoV-2 is affected by mutations in the viral gene, such as the abnormal gene amplification.

The ROK's COVID-19 confirmation diagnostic test method established a test system using the highly sensitive and specific genetic detection test (RT-PCR), and recommended the use of a test method that detects two or more SARS-CoV-2 specific genes [5]. Currently, there are 26 diagnostic test kit products based on the gene detection test (RT-PCR) approved by the Ministry of Food and Drug Safety (MFDA) (as of July 1, 2021, Table 1).

Table 1. Characteristics of COVID-19 detection kits which have been approved by the Ministry of Food and Drug Safety in the Republic of Korea (July 1, 2021)

	Company	Product name	Target gene			
			RdRP	E	N	S
1	SD Biosensor, INC	STANDARD M nCoV Real-Time Detection kit	0	0	-	-
2	BioSewoom Inc.	Real-Q 2019-nCoV Detection Kit	0	0	-	-
3	SEASUN BIOMATERIALS Inc.	U-TOP COVID-19 Detection Kit Plus	0	0	0	0
4	Cancerrop	Q-Sens® COVID-19 Detection kit	0	0	-	-
5	Kogenebiotech	PowerChek⊤SARS-CoV-2, Influenza A&B Multiplex Real-time PCR Kit	0	0	-	-
6	SEASUN BIOMATERIALS Inc.	AQ-TOP COVID-19 Rapid Detection Kit Plus	0	-	0	-
7	Kogenebiotech	PowerChekrSARS-CoV-2 Real-time PCR Kit	0	0	-	-
8	Seegene	Allplex SARS-CoV-2 Assay	0	0	0	0
9	Cancerrop	Q-Sens® COVID-19 Detection Kit V2	0	0	-	-
10	BioSewoom Inc.	Real-Q Direct SARS-CoV-2 Detection Kit	0	0	-	-
11	Seegene	AllplexrSARS-CoV-2/FluA/FluB/RSV Assay	0	-	0	0
12	SML Genetree	Ezplex® SARS-CoV-2 Kit	0	-	0	-
13	Biocore	BioCore 2019-nCoV Real Time PCR Kit	0	-	0	-
14	LabGenomics	LabGunTM COVID-19 ExoFast RT-PCR Kit	0	-	0	-
15	OPTOLANE Technologies Inc	Dr. PCR rDi20K COVID-19 Detection kit	0	0	-	-
16	SEASUN BIOMATERIALS Inc.	U-TOPrSARS-CoV-2 & Flu A/B	0	-	0	-
17	OSANG Healthcare	GeneFinder COVID-19 Fast RealAmp Kit	0	0	0	-
18	BioSewoom Inc.	Real-Q SARS-CoV-2/FluA/FluB Detection Kit	0	0	-	-
19	Bioneer Co	AccuPower® RV1 Real-Time RT-PCR Kit (RV1-1111)	0	0	0	-
20	Roche diagnostics	cobas SARS-CoV-2 & Influenza A/B	0	0	-	-
21	PaxGenBio Co	PaxView® SARS-CoV-2 real-time RT-PCR Kit	0	-	0	-
22	Bioneer Co	AccuPower® RV1 Real-Time RT-PCR Kit (RV1-2112)	0	0	0	-
23	Genomictree	AccuraDtectTM COVID-19 RT-qPCR Kit	0	-	0	-
24	SML Genetree	Ezplex® SARS-CoV2/RV Fast Kit	0	-	0	-
25	AMSBIO	A+CheQ COVID-19 RT-qPCR Detection Kit	0	_	0	_
26	Genematrix	NeoPlexTM FluCOVID Kit	0	-	0	-

As a result of reviewing each product, it was confirmed that most products target the amplified target gene site E and RdRp (ORF1b site), and nucleocapsid (N) proteins, not the gene site of the spike (S) protein. Therefore, the variants does not affect the confirmation. In the case of the three products, the spike (S) gene site, which is the site of mutation, is targeted, but other gene sites are also confirmed to be identified at the same time, so it is unlikely to affect the confirmation.

For example, the AllplexTM SARS-CoV-2 Assay from Seegene. Co., Ltd. target gene is RdRp, E, N, and S, and the same fluorescent material is used to check whether the RdRp gene and S gene are amplified. Therefore, even if the S gene is mutated and only the RdRp gene is amplified, there is no obstacle to being confirmed positive (the premise is that both E and N genes are amplified). In the case of the U-TOPTM COVID-19 Detection Kit Plus from Sisun Biometrics Co., Ltd., four target genes are used in the same way as the Seegene kit, but the reporter dye, which checks whether the gene is amplified, is different from that in the Seegene kit. If the variant of S site leads to no amplification, the result is undecided. In this case, the concentration of the sample is increased according to the manufacturer's manual, and if the same result is obtained after the re-examination, it is determined to be positive (but, verification is needed by sequencing), so it is unlikely that a false negative is determined due to variation.

Therefore, COVID-19 domestic approved diagnostic reagent products are very unlikely to be affected by the diagnosis due to four major mutant viruses (alpha, delta, gamma, and delta), so it is believed that there will be no problem detecting mutant viruses with the current COVID-19 diagnostic system in the ROK. In the future, the Korea Centers for Disease Control and Prevention (KDCA) plans to continuously monitor domestic diagnostic reagent products in preparation for the impact of the COVID-19 variant virus on diagnostic tests.

Acknowledgment

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Submitted: August 19, 2021; Revised: August 23, 2021; Accepted: August 24, 2021

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This article has been translated from the Public Health Weekly Report (PHWR) volume 14, Number 35, 2021.