



# Prevalence of a Single-Nucleotide Variant of SARS-CoV-2 in Korea and Its Impact on the Diagnostic Sensitivity of the Xpert Xpress SARS-CoV-2 Assay

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The sensitivity of molecular diagnostics could be affected by nucleotide variants in pathogen genes, and the sites affected by such variants should be monitored. We report a single-nucleotide variant (SNV) in the nucleocapsid (*N*) gene of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), i.e., G29179T, which impairs the diagnostic sensitivity of the Xpert Xpress SARS-CoV-2 assay (Cepheid, Sunnyvale, CA, USA). We observed significant differences between the threshold cycle (Ct) values for envelope (*E*) and *N* genes and confirmed the SNV as the cause of the differences using Sanger sequencing. This SNV, G29179T, is the most prevalent in Korea and is associated with the B.1.497 virus lineage, which is dominant in Korea. Clinical laboratories should be aware of the various SNVs in the SARS-CoV-2 genome and consider their potential effects on the diagnosis of coronavirus disease 2019.

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**Key Words:** Coronavirus disease 2019 (COVID-19), Diagnosis, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Single nucleotide variant, Xpert Xpress SARS-CoV-2 assay, Performance, Diagnostic sensitivity, Korea

Several PCR primers and probes have been developed for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and various genetic variants have been reported [1-3]. The nucleocapsid (*N*) gene is a common hotspot for single-nucleotide variants (SNVs), and this has raised concerns about its potential effects on diagnostic sensitivity [1-6]. We report a SNV in *N* gene that affects the sensitivity of the Xpert Xpress SARS-CoV-2 assay (Cepheid, Sunnyvale, CA, USA). We further evaluated the emergence of this SNV in Korea and found that it is associated with a specific lineage.

The diagnostic performance of the Xpert Xpress assay was retrospectively validated using SARS-CoV-2-positive clinical nasopharyngeal swabs collected from 10 subjects at Seoul Medical Center, Seoul, Korea (Table 1). All specimens were collected in the viral transport medium and were confirmed to be positive by real-time reverse transcription (rRT)-PCR performed using the STANDARD M nCoV Real-Time Detection kit (SD Biosensor, Suwon, Korea) according to the manufacturer's protocol, and stored at 4°C, from 10 days up to 4 months. As viral RNA is not considered human biological material according to the Enforcement Decree of the Bioethics and Safety Act in Korea, this study was outside the scope of review by an institutional review board and was, therefore, exempted from the requirement of obtaining informed consent from the subjects.

Five specimens were tested using the Xpert Xpress SARS-CoV-2 assay according to the manufacturer's protocol. Signifi-

cant differences were observed between the threshold cycle (Ct) values for *E* and *N* (Table 1, specimens #6–#10). Particularly, in three specimens, the Ct values for *E* and *N2* differed by 10 cycles, and in the other two specimens, *N2* was not detected. The five specimens were retested using both methods, and similar results were obtained.

As nucleotide variants in *N* may account for the observed Ct value discrepancies, we analyzed the five specimens by Sanger sequencing. No information is available on the exact region in *N* that is targeted by the Xpert Xpress primers and probes. However, previous reports suggest that the Xpert Xpress assay employs the *N2* primer set from the SARS-CoV-2 real-time PCR assay of the United States Centers for Disease Control and Prevention (CDC) [8, 9]. A new primer set (forward, AACACAAGCTTTCGGCAGAC; reverse, TGCAGCAGGAAGAAGAGTCA) was designed to amplify the entire *N2* region evaluated in the CDC protocol, with an estimated amplicon size of 385 bp. Sanger sequencing revealed a G>T substitution at position 29,179 (G29179T GenBank: MN908947.3) in all five specimens (Fig. 1). G29179T is located in the 3'-end of the *N2* forward primer of the CDC SARS-CoV-2 PCR protocol [10]. No additional SNV was identified in the five specimens.

To confirm that the SNV identified was responsible for the Ct discrepancies, we analyzed five randomly chosen SARS-CoV-2-positive nasopharyngeal swabs (confirmed between March and April 2020 and stored at -70°C) using the Xpert Xpress assay and Sanger sequencing (Table 1, specimens #1–#5). The Ct value discrepancies between *E* and *N* were less than four cycles, and Sanger sequencing revealed a G at position 29,179 in each specimen. No other SNV was identified in these additional specimens. These results suggested that G29179T was responsible for the Ct value discrepancies between *E* and *N*.

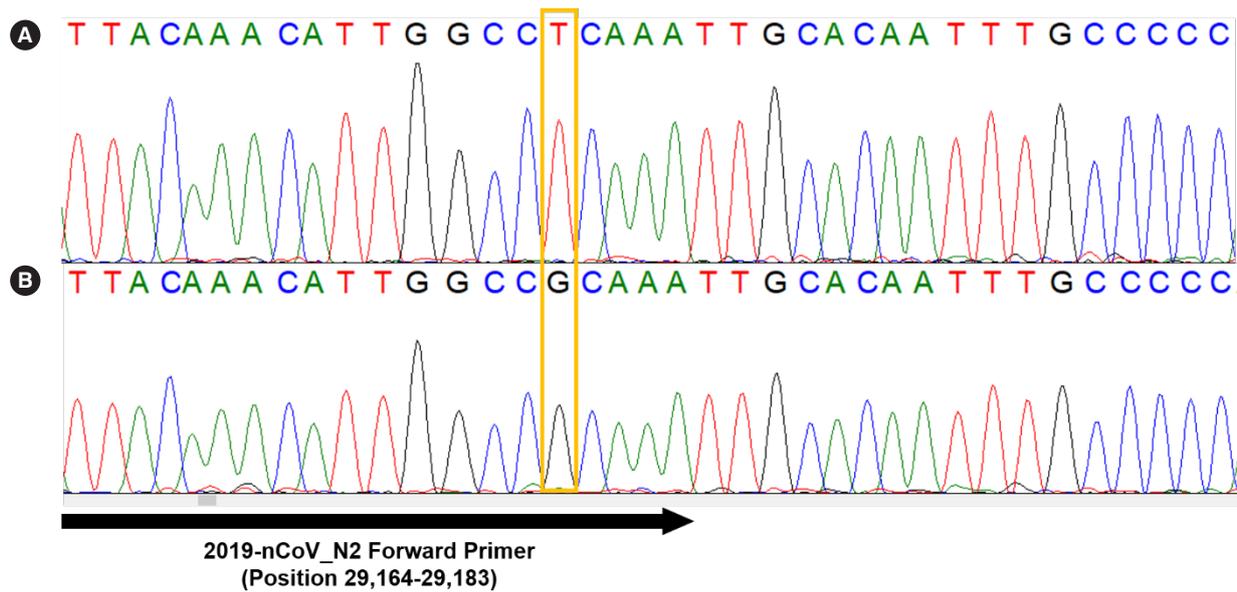
As the exact primer and probe sequences that the Xpert Xpress assay employs are not known—these could be the same, similar, or different from those of the CDC *N2* primers—this SNV may differently affect the diagnostic performance of these two methods. However, Ziegler, *et al.* recently reported a similar phenomenon with the Xpert Xpress assay, which was caused by a different SNV, C29200T, in the *N2* region of the CDC primers [7].

To estimate the prevalence of G29179T in Korea, we analyzed 2,489 SARS-CoV-2 sequences from isolates collected in Korea up to February 28, 2021 and stored in the Global Initiative on Sharing All Influenza Data (GISAID) database [1]. In total, 1,740 sequences (69.9%) harbored G29179T. Most G29179T strains belonged to the PANGO lineage B.1.497 (Table 2, Supplemental Data Fig. S1) [11]. Additionally, G29179T has been reported in

**Table 1.** Results of rRT-PCR\* and Xpert Xpress SARS-CoV-2 assays

Clinical specimen number	Date of confirmation	Result (Ct)			
		rRT-PCR		Xpert Xpress SARS-CoV-2 Assay	
		<i>E</i>	<i>RdRp</i>	<i>E</i>	<i>N2</i>
1	01-Mar-20	30.2	33	30.4	33.6
2	03-Mar-20	26.3	27.5	26.3	28.5
3	27-Mar-20	30	31.9	30.7	34.2
4	01-Apr-20	27.6	29.5	29.4	32
5	24-Apr-20	33.5	33.3	33.7	37.6
6	20-Sep-20	26.2	25.6	27.5	39.3
7	21-Sep-20	32.2	32.7	33.5	44.3
8	22-Sep-20	26.8	26.5	28.7	40.8
9	22-Sep-20	33	32.8	33.5	ND
10	26-Sep-20	29	29.1	30.6	ND

\*STANDARD M nCoV Real-Time Detection kit (SD Biosensor, Suwon, Korea). Abbreviations: Ct, threshold cycle; *E*, envelope gene; *N*, nucleocapsid gene; ND, not detected; *RdRp*, RNA-dependent RNA polymerase gene; rRT-PCR, real-time reverse transcription PCR.



**Fig. 1.** Sanger sequencing results around position 29,179 in the *N* gene of (A) clinical specimen #5 and (B) clinical specimen #8. Abbreviation: N, nucleocapsid.

**Table 2.** Emergence of G29179T in SARS-CoV-2 isolates collected in Korea

Collection	G29179	T29179	
		B.1.497*	Others
Jan 2020	8		
Feb 2020	132		
Mar 2020	105		
Apr 2020	23		
May 2020	30	172	
Jun 2020	40	224	
Jul 2020	128	124	4
Aug 2020	51	242	2
Sep 2020	28	94	2
Oct 2020	40	126	
Nov 2020	14	239	3
Dec 2020	26	266	3
Jan 2021	71	136	1
Feb 2021	53	102	
Sum	749	1,725	15

\*PANGO lineage.

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Peru, Israel, Hong Kong, the United States, Congo, and several other countries, although the prevalence in these countries was significantly lower than that in Korea (Supplemental Data Fig. S2).

The B.1.497 lineage is reported nearly exclusively in Korea

and has been designated as a South-Korean lineage [12]. The SNVs that define B.1.497 include C241T, C1059T, C11916T, C14408T, C16560T, A20675T, A23403G, G25563T, and G29179T. This lineage was first reported in Korea on May 5, 2020, and its prevalence has increased rapidly (Table 2) [1]. Although nearly 70% of GISAID sequences from Korea harbor G29179T, genomic surveillance data to accurately estimate the prevalence of B.1.497 in the country are lacking. However, currently, B.1.497 appears to be a predominant lineage in Korea. This finding highlights the need for more extensive genomic surveillance of SARS-CoV-2 in Korea.

The *E* gene was detected in all specimens. However, in specimens with a low viral load, such as specimens #9 and #10 (Table 1), amplification of *E* could also be interpreted as a negative result. To date, no issue regarding the diagnostic performance of the Xpert Xpress assay with respect to *E* has been reported. However, Artesi, *et al.* reported a SNV in *E* that causes false-negative results in the Roche Cobas SARS-CoV-2 Test (Roche, Basel, Switzerland) [2]. Additionally, some B.1.497 strains reported in the GISAID database harbor SNVs, such as C26313A, in *E* [1]. These findings may suggest that some SNVs affecting the detection of *E* may arise within B.1.497 lineage genomes.

The limitation of our study is that we did not estimate the exact prevalence of B.1.497 lineages, owing to the retrospective nature of this work.

In conclusion, a SNV in *N* was found to affect the sensitivity of the Xpert Xpress SARS-CoV-2 assay. The SNV identified, G29-

179T, is the most prevalent in Korea and is associated with the B.1.497 lineage, which is dominant in Korea. Laboratories should be aware of various SNVs in the SARS-CoV-2 genome and their potential effects on the diagnosis of COVID-19.

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## AUTHOR CONTRIBUTIONS

Hong KH and Lee H take responsibility for the integrity and accuracy of the data. In JW, Lee KA, Kim SH, An YI, and Lee DG performed the PCR and sequencing experiments and analysis. Lee J and Kim SY performed the phylogenetic analysis. Sung H and Kim JS contributed to the revision of the manuscript and the experimental design. All authors have read and approved the final manuscript.

## CONFLICTS OF INTEREST

None declared.

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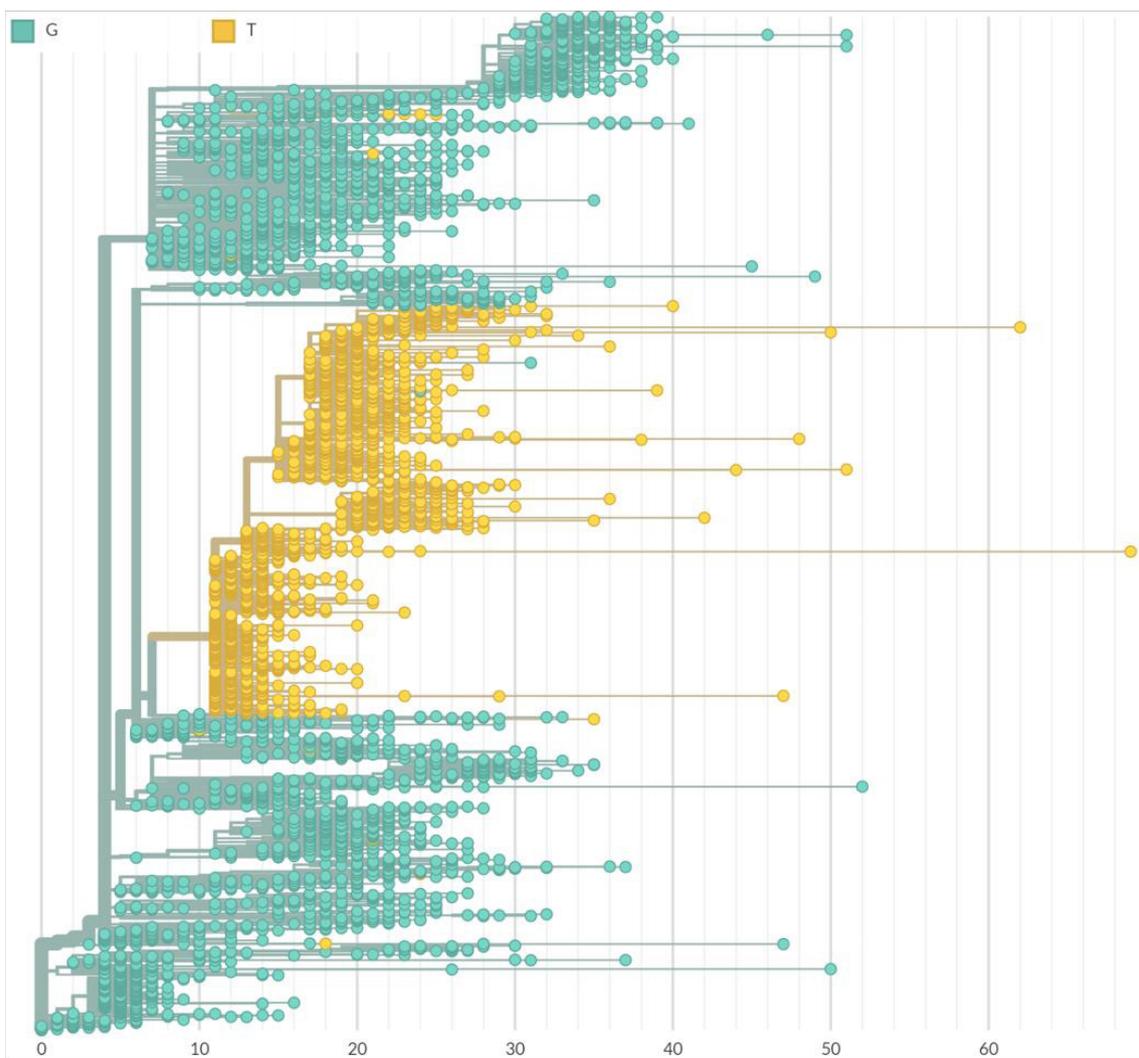
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## Nucleotide at position 29,179



**Supplemental Data Fig. 1.** Phylogenetic tree of 2,489 SARS-CoV-2 sequences from isolates collected in Korea (up to February 28, 2021), generated using Nextclade version 0.7.6 (<https://staging.clades.nextstrain.org>). The presence of G or T at position 29,179 is color-coded as indicated in the figure.

Abbreviations: G, guanine; T, thymine; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.



**Supplemental Data Fig. 2.** Global distribution of the G29179T variant (as of February 28, 2021). The analysis was performed using Nextstrain (<https://nextstrain.org>). Abbreviations: A, adenine; G, guanine; T, thymine.