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LETTER TO THE EDITOR

Endemic SARS-CoV-2 Polymorphisms Can Cause a Higher Diagnostic Target Failure Rate than Estimated by Aggregate Global Sequencing Data

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to mutate during the ongoing COVID-19 pandemic, and some of the nucleotide polymorphisms may result in diagnostic detection failures. Documented polymorphisms resulting in partial failure of diagnostic assays include g.26340C>T resulting in E gene target failure on the Roche (Indianapolis, IN) cobas 6800/8800 assay (1), g.21765_21770 deletion resulting in S gene target failure (SGTF) on the Thermo Fisher (Carlsbad, CA) TaqPath assay that is associated with the B.1.1.7 and other lineages (2, 3), and g.29200C>W resulting in N gene target failure (NGTF) on the Cepheid Xpert assays (4, 5).

In March 2021, the U.S. Food and Drug Administration (FDA) reported that SARS-CoV-2 with an N gene point mutation may cause NGTF when using the N2 primers and probes in the Xpert Xpress SARS-CoV-2, Xpert Xpress SARS-CoV-2 DoD, and Xpert Omni SARS-CoV-2 assays (6). NGTF with Xpert has been described in two *in vitro* reports and associated with translationally silent mutations in the N2 probe region: g.29200C>A and g.29200C>T (4, 5). We investigated the potential for NGTF in contemporary SARS-CoV-2 variants circulating in Northeast Ohio. We report that a synonymous g.29197C>T polymorphism produces NGTF similar to the g.29200C>W polymorphisms, and this g.29197C>T mutation was present in about one-fifth of SARS-CoV-2 genomes that our laboratory has sequenced.

Sixteen unique specimens with SARS-CoV-2 polymorphisms identified near the g.29200 position were selected by convenience sampling and evaluated with Xpert Xpress SARS-CoV-2. The viral polymorphisms were identified by whole-genome sequencing (COVIDSeq; Illumina, San Diego, CA). These 16 upper respiratory swab specimens were collected in March and April of 2021, and SARS-CoV-2 was detected using standard-of-care testing (e.g., TaqPath, Hologic [Marlborough, MA] Panther, and Roche cobas 8800). These specimens included 3 with g.29194T>C, 11 specimens with g.29197C>T, and 2 specimens with g.29200C>T. All of these polymorphisms are translationally synonymous, and therefore, these changes are not expected to impact the proteins translated by the virus.

The g.29194T>C did not significantly impact target detection as the N2 probe was detected approximately 3 cycles after the E gene probe, which is consistent with assay performance in an unmutated genome (7). The 13 samples with g.29197C>T or g.29200C>T had partial or complete NGTF, resulting in a positive or presumptive positive interpretation, respectively, by the Xpert Xpress SARS-CoV-2 assay. The N2 probe was detected approximately 14 cycles after the E gene probe in six samples, and the N2 probe was undetected in the other seven samples (Table 1).

In contrast to the global sequencing data in which the g.29197C>T mutation is present in less than 1% of sequences (internal Cepheid data derived from NCBI and

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TABLE 1 Sixteen samples with synonymous mutations investigated for potential N gene target failure using Xpert Xpress SARS-CoV-2

GISAID accession no.	Lineage	Clade	E[a]	N2[a]	g.29194T>C	g.29197C>T	g.29200C>T
EPI_ISL_1575361	B.1.2	20G	25.2	27.9	x		
EPI_ISL_1563712	B.1.596	20G	23.3	26.4	x		
EPI_ISL_1541185	B.1.596	20G	20	22.7	x		
EPI_ISL_1541162	B.1.1.519	20B	26.8	40.9		x	
EPI_ISL_1541186	B.1.1	20B	28.6	ND[b]		x	
EPI_ISL_1585891	B.1.1.519	20B	28.3	42.1		x	
EPI_ISL_1541164	B.1.1.519	20B	26.7	ND		x	
EPI_ISL_1541187	B.1.1	20B	30.2	43.7		x	
EPI_ISL_1541165	B.1.1.519	20B	33.3	ND		x	
EPI_ISL_1541166	B.1.1.519	20B	27.3	43.7		x	
EPI_ISL_1541170	B.1.1.519	20B	16.1	ND		x	
EPI_ISL_1541171	B.1.1.519	20B	28.4	44.3		x	
EPI_ISL_1541172	B.1.1.519	20B	25.7	ND		x	
EPI_ISL_1541173	B.1.1.519	20B	18.3	ND		x	
EPI_ISL_1575402	B.1.2	20G	14.8	ND			x
EPI_ISL_1575358	B.1.2	20G	27	41.2			x

[a]Cycle threshold at which probe fluorescence was detected by PCR.
[b]ND, not detected.

GISAID databases), our local SARS-CoV-2 sequencing data identified that the g.29197C>T mutation was frequently encountered. The g.29197C>T was identified in 18% (103/567) of all samples sequenced. Eighteen percent (101/567) of the sequenced samples were selected for sequence analysis due to SGTF identified when using TaqPath in standard-of-care testing, and none of these samples with SGTF contained g.29197C>T. However, g.29197C>T was present in 90% (44/49) of samples from the B.1.1.222 lineage and 100% (52/52) of samples from the B.1.1.519 lineage.

The frequency of variants or synonymous mutations of SARS-CoV-2 often varies with geography. In our locale, we have estimated that approximately 20% of positive specimens may contain a g.29197C>T mutation that decreases the ability of the Xpert assay probe to detect the N2 target, but the E gene target is still detectable. Cepheid is actively engaged in updating its assay to accommodate diversity in the SARS-CoV-2 nucleocapsid gene. Clinical, public health, and research laboratories should consider the potential impact of local genomic variation on the performance of the diagnostic assays in use for SARS-CoV-2 detection.

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