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BRIEF REPORT

S-Variant SARS-CoV-2 Lineage B1.1.7 Is Associated With Significantly Higher Viral Load in Samples Tested by TaqPath Polymerase Chain Reaction

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(See the Editorial Commentary by Lemieux and Li, on pages 1663–5.)

A SARS-CoV-2 variant B1.1.7 containing mutation Δ69/70 has spread rapidly in the United Kingdom and shows an identifiable profile in ThermoFisher TaqPath RT-qPCR, S gene target failure (SGTF). We analyzed recent test data for trends and significance. Linked cycle threshold (Ct) values for respiratory samples showed that a low Ct for ORF1ab and N were clearly associated with SGTF. Significantly more SGTF samples had higher inferred viral loads between 1×10^7 and 1×10^8. Our conclusion is that patients whose samples exhibit the SGTF profile are more likely to have high viral loads, which may explain higher infectivity and rapidity of spread.

Keywords. SARS-CoV-2; COVID-19; RT-PCR; TaqPath; viral load; Ct values; Δ69/70; S-variant; B.1.1.7; VOC-202012/01; SGTF; S gene dropout.

The UK response to the SARS-CoV-2 pandemic has involved the setting up of high-throughput diagnostic centers [1] operationally standardized using commercial reverse-transcriptase qualitative polymerase chain reaction (RT-qPCR) testing [2]. The ThermoFisher TaqPath test coamplifies 3 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral gene targets (open reading frame 1ab [ORF1ab], nucleocapsid [N], and spike [S]) from a single clinical sample. Test accuracy is verified between centers using the Qnostics external quality assurance (EQA) panel [3] and subsequent quality assurance by UK External Quality Assessment Services [4], and has National Health Service England/Improvement accreditation for the routine diagnostic service.

In December 2020, UK authorities were alerted to the emergence of a SARS-CoV-2 variant of genetic lineage B1.1.7 [5], synonymous with the variant under investigation (VOC-202012/01) by Public Health England, which appeared to be spreading rapidly through the United Kingdom [6]. The variant possesses several mutations, of which the Δ69/70 deletion results in failure to detect the S gene target (SGTF) of the TaqPath test, with the other 2 gene targets ORF1ab and N not affected. We undertook to review past positive results for evidence that the Δ69/70 variant had been present in these respiratory samples, that overall test accuracy had still been maintained, and investigated its relationship with the other viral gene targets.

METHODS

The data used for this analysis are RT-qPCR cycle threshold (Ct) values originating from laboratory testing of respiratory samples in the UK Department of Health and Social Care Test and Trace network. A dataset of 641 SARS-CoV-2–positive results received during the period 25 October to 25 November 2020 was used for this analysis (available in [7]).

All positive results had amplifiable bacteriophage MS-2 internal control, with no evidence of general inhibition in the RT-PCR reaction. Raw Ct values were analyzed with respect to the presence of ORF1ab, N, or S gene single-target signals. For the purposes of this analysis, gene target-negative signals were separated into (1) target signal detected but above the threshold of Ct 37, as stipulated for clinical interpretation of results in the TaqPath instructions for use, and (2) targets having no signal detected, which were assigned a nominal Ct value of 45. This latter group of S gene target failures are defined as SGTF.

Frequency comparisons, χ^2, and Mann-Whitney *U* tests for significance of non-Gaussian distributions between SGTF and S gene-positive RT-qPCR results were performed using GraphPad Prism version 5.03.

The process of inferring relative viral loads was based on the laboratory performance for the Qnostics EQA panel results (Figure 1A), which has quantitative information relating copies per mL of whole-virus lysate derived from cell culture of SARS-CoV-2. Relative viral loads were inferred by standard efficiency calculations of RT-PCR tests, where a 3.3-Ct difference between targets approximates to a 10-fold change in substrate.

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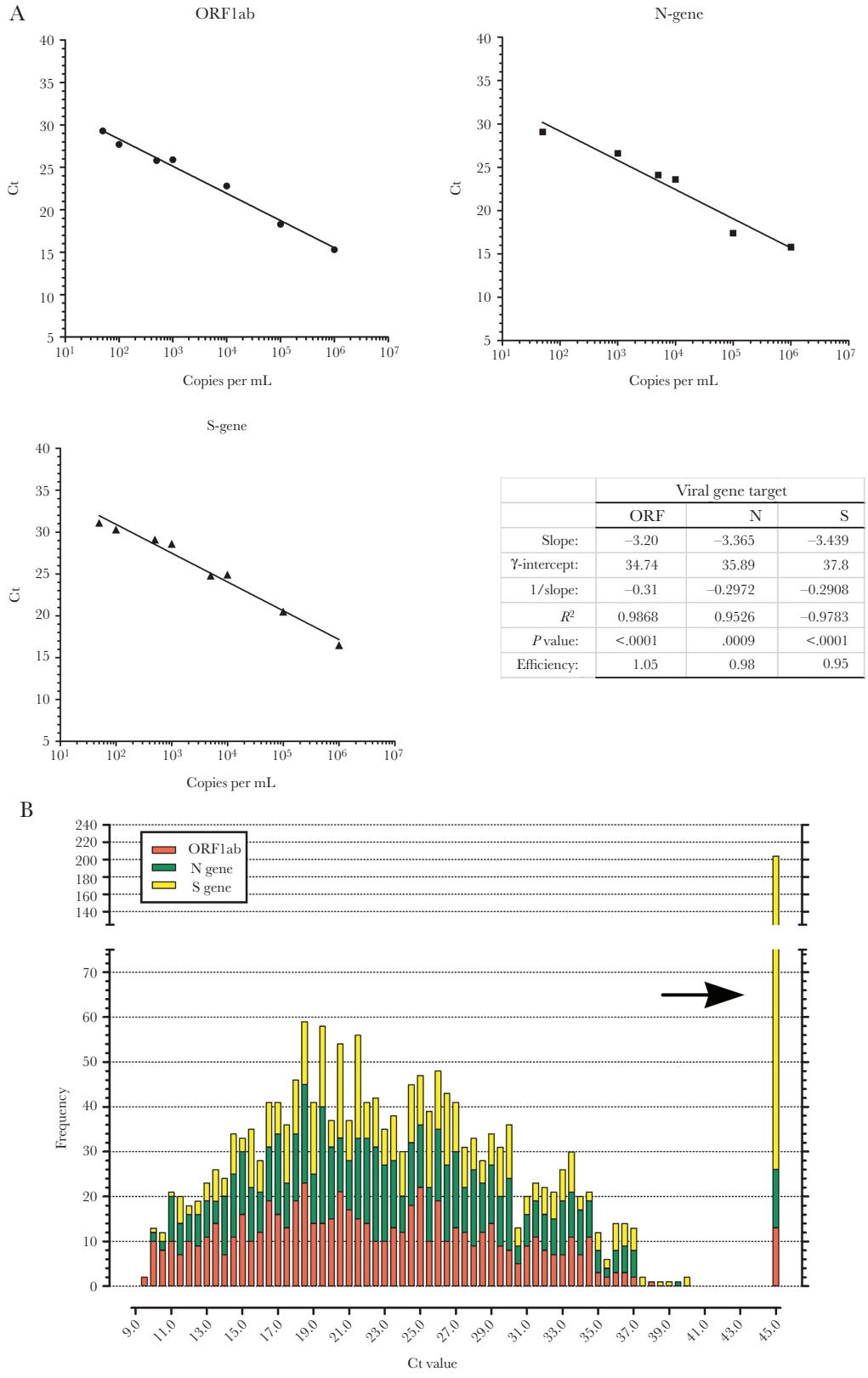

	Viral gene target		
	ORF	N	S
Slope:	−3.20	−3.365	−3.439
Y-intercept:	34.74	35.89	37.8
1/slope:	−0.31	−0.2972	−0.2908
R^2	0.9868	0.9526	−0.9783
P value:	<.0001	.0009	<.0001
Efficiency:	1.05	0.98	0.95

Figure 1. *A*, Verification data for TaqPath RT-PCR test using the Qnostics external quality assurance dilution series SCV2AQP01-A. Linear regression plots for ORF1ab, N, and S gene targets are shown with regression data including efficiency calculations for each gene target. *B*, Stacked bar frequency diagram showing the proportional relationship between all 3 viral gene targets detected by RT-qPCR, at each Ct value across the range. Y-axis is divided into a low sample frequency range of 0–75 and a high range of 130–240. Arrow shows gene target failures for S, N, and ORF1ab. Abbreviations: Ct, cycle threshold; N, nucleocapsid; ORF1ab, open reading frame 1ab; RT-qPCR, reverse transcription quantitative polymerase chain reaction; S, spike.

RESULTS

Frequency Analysis for All 3 Gene Targets

Figure 1B shows a significantly higher proportion of SGTF samples (178 of 641; 27.7%), far right at Ct 45 (indicated by arrow), compared with either ORF or N gene undetectable positive profiles (both 13 of 641; 2.0%; χ^2 [1, n = 641] = 165.46, $P < .00001$). Further nonstatistical observation suggests that for lower Cts (9–22), in each bar the S gene appears at a lower frequency than the corresponding ORF1ab and N genes. At Cts from 25 to 33 and onwards, this trend is less obvious with similar frequencies of all 3 viral genes detected. In particular, towards the limit of the test sensitivity range (Ct 35–40), ORF1ab, N, and S are detected at approximately equal frequencies with no apparent consistent loss of sensitivity for any gene target.

SGTF Samples Had Significantly Lower Median Ct of Corresponding ORF1ab and N Gene Targets

To determine whether, on a population basis, SGTF samples were significantly associated with lower Ct values of either ORF1ab or N gene target in positive samples, the distribution of SGTF and S-detected samples was compared within all ORF1ab- and N-positive samples (Figure 2A). In both, the median Ct value of SGTF and S detected was significantly different (ORF1ab gene 18.16 vs 22.30; N gene 19.39 vs 23.16; both $P < .0001$, Mann-Whitney *U*). Clustering of SGTF results around very low Ct values of ORF1ab and N can be clearly observed, probably accounting for the lowering of the group median Ct.

Linked Ct Values for Individual Samples Show a Propensity for SGTF Samples to Be Associated With a Lower Ct for ORF1ab and N Gene Targets

Because it is possible that a low Ct of ORF1ab or N gene targets in SGTF could occur in different samples, we further investigated the likelihood for the SGTF profile to be associated with low Ct values of both other viral targets in the same sample (Figure 2B). Such SGTF profiles appeared more likely to be associated with low Ct, and a χ^2 analysis of the number of samples below a Ct value of 15 in both categories of SGTF (63/178; 35.4%) and non-SGTF (46/450; 10.2%) was performed for the ORF-positive group. Results showed a highly significant difference between proportions (χ^2 [1, n = 628] = 36.61, $P < .0001$). Therefore, our observed cluster of SGTF samples corresponds to a significantly larger population of infectious subjects having an increased viral load, which can be up to 10 000-fold higher than the non-SGTF median (Ct 9 vs Ct 23). Conservative extrapolation from the upper end of the Qnostics EQA panel data suggests that the larger population has viral loads of between 1×10^7 (approximately Ct 12) and 1×10^8 (approximately Ct 9) copies per mL.

DISCUSSION

The SARS-CoV-2 variant of concern (VOC-202012/01; Public Health England) has spread rapidly throughout the south-east of the UK, and latterly to other regions [6]. More detailed published evidence shows that the presence of the Δ69/70 mutation in the viral genome, causing the SGTF phenomenon in TaqPath RT-qPCR tests [8], strongly correlates [9] with presence of the VOC/B1.1.7 in clinical samples as determined by sequencing [10], and is now used as an epidemiological proxy for presence of the variant.

We analyzed positive results from samples submitted to the Birmingham Turnkey laboratory between 25 October and 25 November 2020, from both local and distant UK geographical areas, at a time where the incidence of VOC/B1.1.7 was increasing sharply [6]. Our analysis shows a high proportion of SGTF, with other gene targets ORF1ab and N being clearly detected in the same sample. Further analysis shows that a significantly higher number of SGTF samples are associated with these lower Ct values of ORF1ab and N, from which it is possible to infer conservatively a high viral load in this larger population of between 1×10^7 and 1×10^8 copies per mL. Although in our comparisons similar viral loads were seen in non-SGTF samples, the significantly higher number of subjects having an extreme viral load is of great concern.

The capability of increased transmission has been ascribed to the VOC: epidemiological tracking, as either its SGTF proxy or sequenced as B1.1.7, has shown that its secondary attack rate is higher than wild-type virus [10] and that it has a significant 50%–75% multiplicative increase in reproductive number compared to non-SGTF variants [11]. Our finding in laboratory data, that a significantly larger proportion of subjects whose samples show SGTF have an inferred viral load at the extreme end of the range, may represent an explanation for this. We recommend that further investigations should include the possibility that either short-term very high viral load or an extension of the period of infectious viral excretion during the symptomatic phase of illness [12] would both increase the likelihood of the virus to transmit onwards and be compatible with our findings.

At a technical level, we considered alternative explanations for SGTF at very low ORF1ab and N Ct values. These include the argument that chemical components in an individual RT-qPCR reaction become limited when amplifying multiple targets at high viral load input, and possibly the S gene target is first to become nonamplifiable. However, the TaqPath test contains an internal control provided by coamplification of non-human bacteriophage MS-2, with the target RNA included at a concentration that is more likely to become undetectable under adverse reaction conditions than any of the 3 specific gene targets, and provides reassurance that amplification of all specific targets is not being inhibited. All positive TaqPath results in the data table were passed as valid, determined by the presence of MS-2 amplification, making it unlikely that SGTF are due to a general reaction chemistry bias.

During the laboratory verification of the TaqPath test, using an EQA standard dilution series derived from cultured

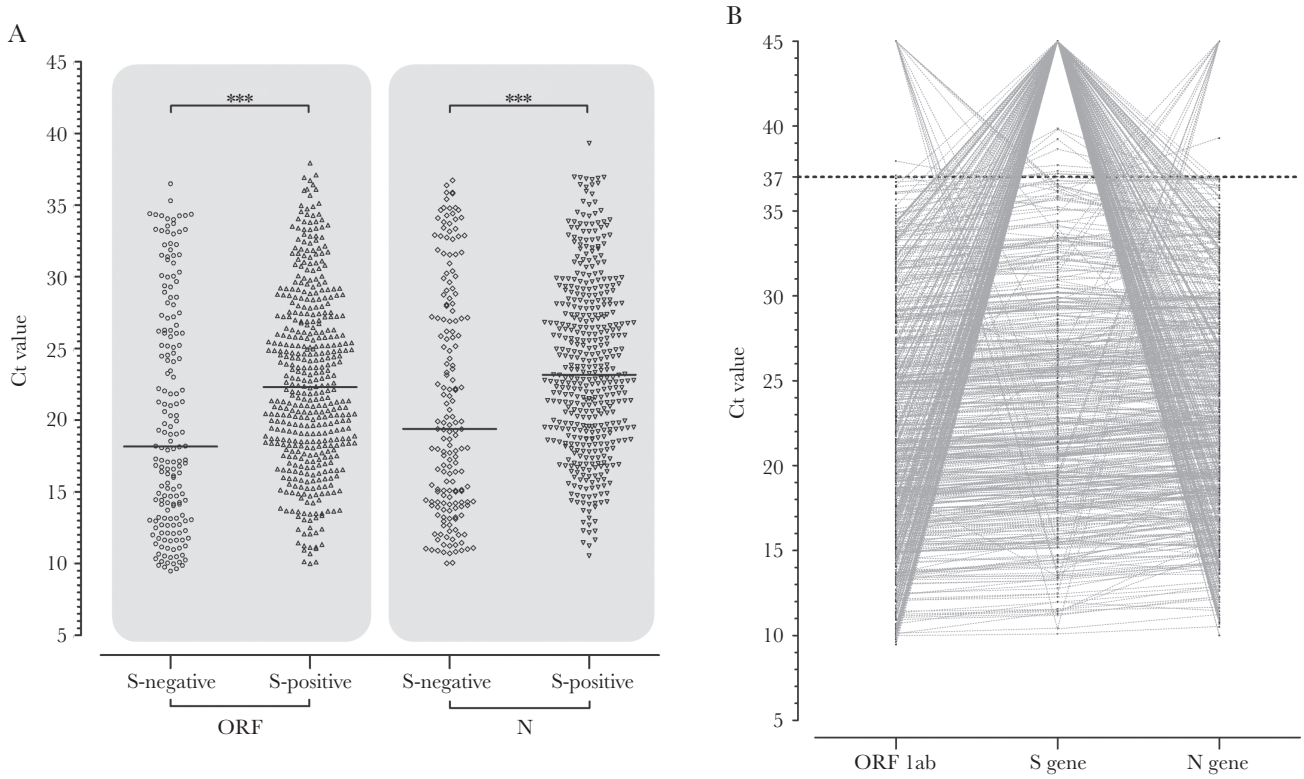

Figure 2. *A*, Scatter plot of the population of S gene-negative (SGTF)/S-gene positive Cts within corresponding ORF1ab and N-gene positive samples. Median Ct is shown by a black horizontal bar. Above both plots the results of Mann-Whitney *U* tests for significant differences are shown; *** $P < .0001$. *B*, Scatter plot of viral gene Ct values for individual positive samples. The horizontal dotted line at Ct 37 represents the threshold for a target-negative result as defined for clinical interpretation in the TaqPath instructions for use. The class of target-undetectable results have a nominal value of Ct 45 assigned (described in "Methods" section). Each set of 3 Ct values for a sample is linked by a grey dotted line. Abbreviations: Ct, cycle threshold; N, nucleocapsid; ORF1ab, open reading frame 1ab; S, spike.

SARS-CoV-2, there were no observed SGTF at Ct levels of approximately 15, which is lower than the median Ct value at which SGTF were seen to occur in our data. This lends further weight to discount the influence of general RT-qPCR reaction inefficiencies in our observations.

Another alternative, but theoretical, explanation for the low Ct of ORF1ab and N with SGTF is that inhibition of S gene amplification results in greater availability of in vitro reagents and less competition for enzymatic activity, which enables preferential increases in ORF1ab and N. However, we believe this is unlikely because the deletion mutation interfering with S gene detection only affects the probe binding, with silent yet authentic coamplification of the S gene target [6]. We have confirmed this to be the case by colleagues sequencing our SGTF samples (personal communication to M. K. from A. M.), in which the S gene amplicons were clearly present and had the Δ69/70 deletion.

It should be emphasized that the authenticity of positive results is not affected by the presence of the SGTF phenomenon, as the TaqPath test result is classed as positive when 2 gene targets are detected. Thus, the ability of the TaqPath test to detect 3 viral targets provides a degree of robustness to the Test and Trace program, even when a viral mutation renders 1 of them undetectable.

As a parallel observation, some types of commercial test for SARS-CoV-2 rely on reactivity to the S gene or its protein product, for example the lateral flow devices. A recent preliminary assessment of the performance of 5 of these tests has shown that they are not affected by the VOC/B1.1.7 [13].

For clarity, we do not anticipate that the mutation causing SGTF is necessarily responsible for higher viral load in patients. There are additional mutations in B.1.1.7 that could have a direct contribution, with SGTF as an indirect marker in the TaqPath RT-PCR for the presence of the B.1.1.7 variant. Whole-genome sequencing of individual samples will prove to be valuable in strengthening the association with changes in the viral genome.

Finally, we also observed dropouts for the ORF1ab and N genes (Figure 1B)—albeit at a much lower frequency and not apparently associated with high viral loads—and we believe these should be similarly investigated for mutations in the corresponding genes that could have affected their detection. We also note that double dropouts—where 2 viral genes are not amplified in a sample—by definition will not be represented in the original data as they would be classed as negative. A more exhaustive analysis would involve reviewing all negative results where a single viral gene was amplified.

Limitations of these data are, firstly, our analysis may provide additional evidence to explain why VOC-202012/01 (B.1.1.7) may be transmitting more rapidly amongst populations, but it does not provide an explanation of how an increased viral load could occur. If verified by others, the biological plausibility of its higher infectivity, whether through evolutionary viral replication advantages or evasion of the host immune system, is yet to be determined. Secondly, although we have made broad inferences in relative viral load in the samples, the TaqPath is not designed as a quantitative assay for SARS-CoV-2 and our observations should be repeated by a dilution series or a validated quantitative method.

Notes

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Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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