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# UTILITY OF S-GENE TARGET FAILURE (SGTF) APPROACH IN THE DETECTION OF OMICRON VARIANT

Medical Microbiology

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## ABSTRACT

**Background:** With the emergence of the SARS-CoV-2 Variant of Concern, B.1.1.529 (Omicron), the World Health Organization, in its technical briefing dated 28th November 2021, had advised the use of the S gene target failure (SGTF) approach as a proxy test for faster screening of Omicron cases, pending confirmation by sequencing. Therefore, the present study aims to study the utility of the SGTF approach as a marker for early detection of the Omicron variant. **Materials and Methods:** A total of 520 RT-PCR positive RNA samples between 1st December 2021 to 22nd February 2022 and with a cycle threshold (Ct) value of less than 25 were tested for SGTF and sequenced to identify the SARS-CoV-2 variant. The results of both tests were entered and analyzed using Microsoft Excel. **Results:** Out of 520 samples sequenced, 43.46% were Omicron sub-lineage BA.2 followed by 22.50% Omicron sub-lineage BA.1, 16.35% Delta sub-lineages (AY.*), 7.88% Delta (B.1.617.2), 6.54% Omicron B.1.1.529, 1.73% Omicron sub-lineage BA.1.1 and 1.54% other lineages. Among the Omicron samples, SGTF was seen in 88.89% of BA.1 and BA.1.1 each, in 52.94% and 3.54% of cases of B.1.1.529 and BA.2, respectively. The sensitivity, specificity, positive, and negative predictive values of the SGTF test for detection of the Omicron variant were 35.75%, 81.34%, 84.66%, and 30.53%, respectively. **Conclusion:** With the dominance of the BA.2 Omicron sub-lineage during the latter half of the study period, the SGTF approach for early screening of Omicron cases lost its significance as BA.2 escaped SGTF detection due to the absence of del 69/70 in its spike gene.

## KEYWORDS

S-gene Target Failure (SGTF), SARS-CoV-2 Whole-Genome Sequencing (WGS), Omicron, B.1.1.529, BA.1, BA.2

## INTRODUCTION

Over the past two years, SARS-CoV-2, the virus causing COVID-19 infection, has evolved to give rise to new variants that are more easily transmitted, are more virulent and are able to evade the immune system. Some of the notable variants of Concern (VOCs) that have emerged recently are Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2).[1] On November 26, 2021, the World Health Organization (WHO) designated the Omicron variant (B.1.1.529) as a Variant of Concern (VOC).[2]

The Omicron variant, a highly divergent variant[2], is currently the dominant variant circulating globally. It accounts for nearly all sequences shared in the Global Initiative on Sharing All Influenza Data (GISAID).[3] Its genome includes 18,621 mutations, with over 97% of them located in the coding region.[4] There are 26-32 mutations, primarily located in the receptor-binding domain of the spike protein.[2,4] The observed mutations may potentially impact the accuracy of diagnostic tests, the effectiveness of treatments, and the effectiveness of vaccines.[5] Therefore, its early and timely detection is of paramount importance.

Whole genome sequencing (WGS) of the SARS-CoV-2 virus is necessary to identify and track emerging mutations. However, it is relatively resource-intensive and can take several days to generate results. This delay in reporting can hinder the public health response and make it difficult to determine the current prevalence of different variants in a community in real time. To address this issue, alternative methods such as nucleic acid amplification-based screening tests that provide results in a few hours can be useful for early detection of Variants of Concern (VOCs).[6] The existing SARS-CoV-2 PCR tests are able to detect the Omicron variant. However, the Thermo Fisher TaqPath COVID-19 Combo Kit, targeting the Spike (S) gene, the Nucleocapsid (N) gene, and Open Reading Frame (ORF), has shown a reduced ability to detect the S-gene due to deletions at positions H69 and V70 in the spike protein. This is known as S-gene Target Failure (SGTF) and was previously used as a marker to identify the Alpha variant.[5]

Therefore, following the WHO guidelines for using the SGTF approach, this study was conducted to assess the utility of this approach in the early detection of the Omicron variant. The study used whole genome sequencing (WGS) as the reference standard for comparison.

## MATERIAL AND METHODS

A total of 520 RT-PCR positive RNA samples with a cycle threshold (Ct) value of less than 25, collected from various collection centers in Pune between 1st December 2021 to 22nd February 2022, were included in the study. The samples were tested for SGTF, and the SARS-CoV-2 variants were characterized using whole-genome sequencing. The present study was conducted at a tertiary care center in Pune, Maharashtra.

### Multiplex Real-Time PCR

The RNA samples were subjected to multiplex real-time PCR using TaqPath COVID-19 Combo Kit (Thermofisher Scientific, USA). The PCR assay was performed using QuantStudio™ 6 Pro Real-Time PCR System (Thermofisher Scientific Inc., Waltham, U.S). The target genes used for detecting SARS-CoV-2 are the Nucleocapsid (N) gene, the Open Reading Frame - 1ab (ORF-1ab) gene and the Spike (S) gene. The amplification data were interpreted based on the cut-off Ct value recommended by the manufacturer. SGTF was defined as failure to detect the S-gene target in samples testing positive for both N-gene and ORF1ab gene targets.

### Whole-Genome Sequencing of SARS-CoV-2 virus

Irrespective of the S-gene amplification results, SARS-CoV-2 whole-genome sequencing of 520 RNA samples was performed. The samples were sequenced using GridION sequencer, Oxford Nanopore Technologies, UK, NextSeq 550 sequencer, Illumina Inc, USA and IONTorrent sequencer, Thermofisher Scientific. Lineage and Clade analysis was done using Phylogenetic Assignment of the Named Global Outbreak LINeages (PANGOLIN) and Nextclade Software.

### Statistical Analysis

The results of both the tests were entered and analyzed using Microsoft Excel. The sensitivity, specificity, positive, and negative predictive values of the SGTF test for detection of the Omicron variant were calculated using Microsoft Excel.

## RESULTS

A total of 520 RT-PCR positive RNA samples were included during the study period, of which 176 (33.85%) were from December 2021, 290 (55.77%) from January 2022 and 54 (10.38%) were from February 2022. Out of the 520 samples, 308 (59.23%) were males, and 212 (40.77%) were females. The mean age of the study population was 33.8 years. Out of the 520 samples tested for SGTF, 163 (31.35%) were positive for SGTF, and 357 (68.65%) were non-SGTF samples. The distribution of SGTF and non-SGTF samples over the study period is shown in Figure 1.

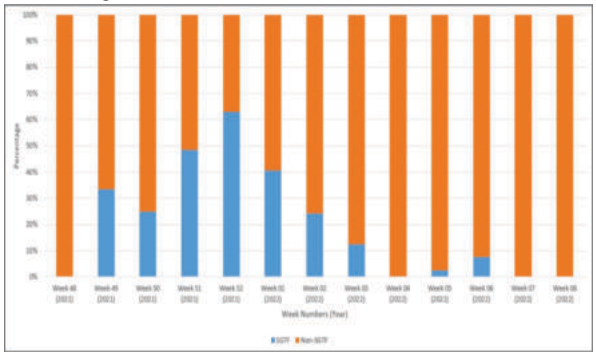

**Figure 1: Distribution of S gene Target Failure (SGTF) and Non-S gene Target Failure (Non-SGTF) over the study period (From 1st December 2021 to 22nd February 2022)**

Similarly, out of the 520 samples sequenced, 386 (74.23%) were identified as VoC Omicron variant and its sub-lineages, followed by 125 (24.04%) VoC Delta variant and its sub-lineages and 09 (1.73%) other lineages. Figure 2 shows the prevalence of different SARS-CoV-2 variants among the samples sequenced during the study period. December 2021 was dominated by Delta sub-lineages (AY.*) (39.2%), followed by Omicron sub-lineage BA.1 (30.68%) and Delta (B.1.617.2) variant (21.02%), whereas in January 2021, Omicron sub-lineage BA.2 was the predominant (59.31%) variant followed by BA.1 (21.38%) and B.1.1.529 (7.93%). In February 2022, 96.30% (52/54) of samples sequenced were BA.2, followed by BA.1 (1.85%) and BA.1.1 (1.85%).

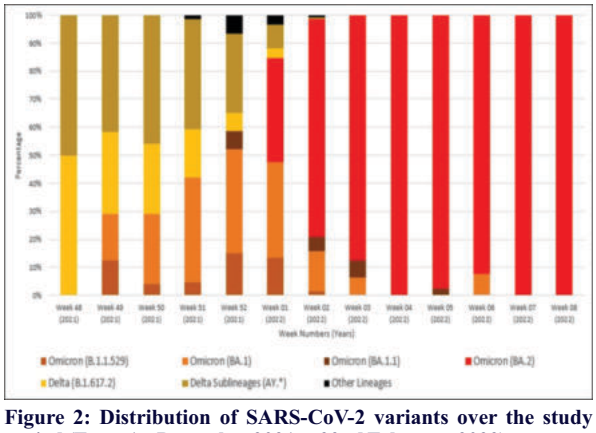


**Figure 2: Distribution of SARS-CoV-2 variants over the study period (From 1st December 2021 to 22nd February 2022)**

Table 1 and Figure 3 show the correlation between SARS-CoV-2 variants and the SGTF results. Among the Omicron variants, SGTF was seen in 88.89% of BA.1 and BA.1.1 each, in 52.94% of B.1.1.529 and only 3.54% of BA.2 cases. Similarly, there were 18/85 (21.18%) Delta sub-lineages showing SGTF. On analyzing the sequences of these Delta sub-lineages, it was found that in 16 AY.* samples, the region of interest in the S-protein had no coverage. Two samples identified as AY.112 and AY.125 had del 69/70 mutation in their S-protein. B.1.1.528 and C.36.3.1 were among other lineages showing SGTF. Two out of four samples positive for SGTF had poor genome coverage and could not be characterized, and the remaining two samples were not assigned any Pangolin lineage.

**Table 1: Correlation between SARS-CoV-2 variants and SGTF results**

| SARS-CoV-2 Variant Name | SGTF | Non- SGTF | Total |
|---|---|---|---|
| Omicron (B.1.1.529) | 18 (52.94%) | 16 (47.06%) | 34 (100%) |
| Omicron (BA.1) | 104 (88.89%) | 13 (11.11%) | 117 (100%) |
| Omicron (BA.1.1) | 08 (88.89%) | 01 (11.11%) | 09 (100%) |
| Omicron (BA.2) | 08 (3.54%) | 218 (96.46%) | 226 (100%) |
| Delta (B.1.617.2) | 01 (2.44%) | 40 (97.56%) | 41 (100%) |
| Delta Sub-lineages (AY.*) | 18 (21.18%) | 67 (78.82%) | 85 (100%) |
| Other Lineages | 02 (50%) | 02 (50%) | 04 (100%) |
| Lineage not assigned/ samples with low coverage | 04 (100%) | 00 (0%) | 04 (100%) |
| Total | 163 (31.35%) | 357 (68.65%) | 520 (100%) |

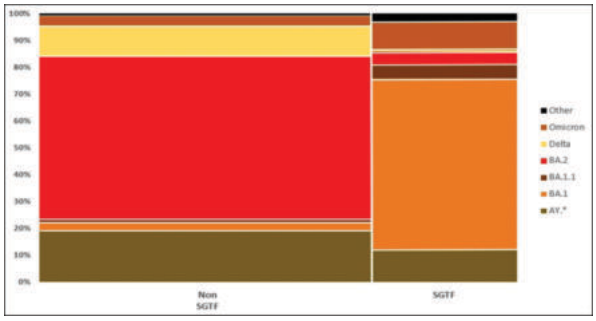


**Figure 3: Mosaic Plot showing the frequency of variants and SGTF status**

The performance characteristics of the SGTF approach in the detection of the Omicron variants are shown in Table 2. The overall sensitivity, specificity, positive, and negative predictive values of the SGTF test for detection of the Omicron variant as a whole were 35.58%, 80.74%, 84.05%, and 30.53%, respectively.

**Table 2: Performance characteristics of the SGTF approach in detection of Omicron variants**

| Characteristics | B.1.1.529 | BA.1 | BA.1.1 | BA.2 |
|---|---|---|---|---|
| Sensitivity | 52.94% (35.13% to 70.22%) | 88.89% (81.75% to 93.95%) | 88.89% (51.75% to 99.72%) | 3.54% (1.54% to 6.86%) |
| Specificity | 70.16% (65.88% to 74.20%) | 85.36% (81.53% to 88.66%) | 69.67% (65.48% to 73.63%) | 47.28% (41.45% to 53.16%) |
| Positive Likelihood Ratio | 1.77 (1.26 to 2.51) | 6.07 (4.76 to 7.75) | 2.93 (2.25 to 3.82) | 0.07 (0.03 to 0.13) |
| Negative Likelihood Ratio | 0.67 (0.47 to 0.96) | 0.13 (0.08 to 0.22) | 0.16 (0.03 to 1.01) | 2.04 (1.80 to 2.31) |
| Positive Predictive Value (*) | 11.04% (8.08% to 14.91%) | 63.80% (57.99% to 69.24%) | 4.91% (3.81% to 6.31%) | 4.91% (2.53% to 9.32%) |
| Negative Predictive Value (*) | 95.92% (93.69% to 96.83%) | 96.36% (94.06% to 97.79%) | 99.72% (98.25% to 99.96%) | 38.94% (36.05% to 41.90%) |
| Accuracy (*) | 69.04% (64.87% to 72.99%) | 86.15% (82.88% to 89.01%) | 70.00% (65.86% to 73.91%) | 28.27% (24.44% to 32.35%) |

## DISCUSSION

The Omicron variant B.1.1.529 was designated as a VOC by WHO on 26th November 2021. With time, it has been further classified into

several sub-lineages, of which BA.1, BA.1.1 and BA.2 are the most common sub-lineages reported.[3] As of 2nd April 2022, the apparent cumulative prevalence of BA.2 worldwide was 6% (with 5,36,303 sequences deposited on the GISAID database), and in India, it was 17% (with 20,217 sequences).[7] Similarly, the worldwide cumulative prevalence of BA.1.1 and BA.1 was 8% (6,86,651 sequences deposited) and 3% (2,80,531 sequences deposited), and in India, it was 2% (2,785 sequences deposited) and 1% (730 sequences deposited), respectively.[8,9]

Throughout the COVID-19 pandemic, the SARS-CoV-2 virus has mutated, resulting in various genetic variations in the circulating virus population. Such mutations impact the performance of diagnostic tests. The performance of a test is influenced by multiple factors the test design, the variant genomic sequence, and the prevalence of a variant in a population.[10] In the present study, the prevalence of SGTF and non-SGTF was influenced by the prevalence of BA.1, BA.1.1, Delta, Delta sub-lineages and BA.2 variant during the study period. Figure 4 shows the prevalence of VOCs SARS-CoV-2 variant over time in India. Apparently, every wave of the SARS-CoV-2 pandemic was driven by a VOC. With the emergence of a fitter variant, all other variants circulating at a given time and place are replaced by the fitter variant.

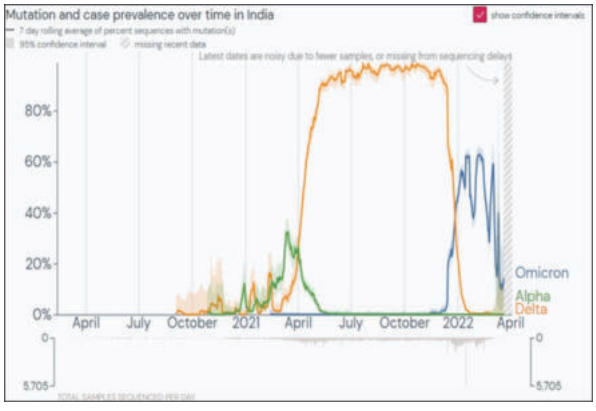


**Figure 4: Prevalence of SARS-CoV-2 variant over time in India. This graph is generated and taken from Outbreak.info.[11]**

The Omicron variant is the most heavily mutated variant known among all the VOCs[4], thus raising concerns about the potential impact of these mutations on its detection by routine RT-PCR tests. Therefore, in its technical briefing dated 28th November 2021, the WHO asked the countries for enhanced surveillance for the Omicron variant using the SGTF approach. It also advised the countries with access to diagnostic tests containing the S gene target to prioritize specimens with SGTF for sequencing to confirm the presence of the Omicron variant.[2]

The deletion at nucleotide position 207-212 (del H69-V70) is responsible for the S-gene target failure.[2] It was previously used as a proxy test for detecting the Alpha variant as del 69-70 is seen in 97% of Alpha variant sequences deposited on GISAID. It is seen in 97% of BA.1, 97% of BA.3 and 96% of BA.1.1 sequences. As of 23rd December 2021, del 69-70 was seen in about half of the sequences submitted on GISAID.[12] It is typically absent in Omicron sub-lineage BA.2. However, del 69-70 is seen in less than 0.5% of BA.2 sequences worldwide.[2] Del 69-70 is seen in 75% of C.36.3.1 sequences and 2% of B.1.1.528 sequences. Among the Delta and its sub-lineages, del 69-70 is seen in less than 0.5% to 2% of sequences worldwide.[13] Thus, non-Omicron variants possessing the del 69-70 may also trigger SGTF. Also, a gene dropout may be observed in the absence of a specific mutation in the target gene. This may occur due to the varied sensitivity of the genetic target.[10] Therefore, samples positive for SGTF should be confirmed by sequencing to characterize the variant and rule out variants other than the Omicron variant circulating at low frequencies in a given population at a given point in time. As per the European Centre for Disease Control, at least a subset of SGTF samples (i.e., minimum of 10%) should be subjected to sequencing to increase the confidence and reliability of the results. In areas where other variants carrying the del 69-70 are circulating, sequencing of all SGTF samples is necessary.[6]

In the present study, the SGTF approach for detection of the Omicron variant lost its significance in the latter half of the study period, as it was dominated by the BA.2 Omicron sub-lineage, which escapes detection by the SGTF approach. However, it was helpful in the detection of other Omicron variants BA.1 and BA.1.1. India's prevalence of BA.2 lineage over the last 60 days is approximately 94%.[11] Therefore, with the rapid spread of BA.2 to various parts of the world, the sequencing of positive samples, irrespective of their SGTF status, will help us closely monitor the SARS-CoV-2 virus.

## CONCLUSION

The SGTF approach combined with SARS-CoV-2 whole-genome sequencing can be used as a proxy for Omicron (B.1.1.529) and its sub-lineages BA.1 and BA.1.1. However, it is no longer helpful in screening the Omicron variant, as SGTF does not detect BA.2 sub-lineage. This approach can be used to assess the correlation of SGTF and specific VOC, as this will vary with the regionally circulating variants. In case of high correlation, SGTF can be used to monitor the frequency of variants possessing deletion in S-gene in the community.

**Conflict Of Interest:** None to declare

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