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Original Research Article

A distinctive genomic feature, the spike gene amplification failure (SGAF), enables accurate detection of the Alpha and Omicron SARS-CoV-2 variants of Gujarat region

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Abstract

Background and Objective: COVID-19 is the disease caused by infectious agent SARS-COV-2. Several mutations in spike gene are shared by the Alpha (B.1.1.7) and Omicron (B.1.1.529, BA.1, BA.4, and BA.5) variants of concern (VOC). The TaqPath RT-PCR kit for SARS-CoV-2, fails to detect the Spike gene target due to Del 69–70. Alpha and Omicron VOCs have been inferred in part from the Spike Gene Amplification Failure (SGAF) marker.
Materials and Methods: Samples of routine COVID-19 RT-PCR testing between January 2021 to August 2022 were considered in this study. RNA was extracted and subjected to RT-PCR by Taqpath kit as well NGS by Ion AmpliSeq SARS-CoV-2 Research Panel. FASTA files were analysed by nextstrain database and statistical analysis were executed by GraphPad Prism version 9.5.0.
Results: 80 **S**amples were processed for NGS including 50 SGAF-detected samples. For the Alpha variant, the SGAF marker's sensitivity and specificity were 99.7% (95% CI 97.3–99.9%) and 99.4% (95% CI 97.4–99.7%), while for the Omicron variant, they were 99.2% (95% CI 98.7–98.9%) and 99.6% (95% CI 99.3–99.7%).
Conclusion: The SGAF's positive predictive value was 100% for Omicron and 98% for Alpha. The high accuracy of the SGAF marker ensures a precise tool for identifying these variants in laboratory conditions. Furthermore, real-world data has demonstrated that SGAF testing aligns closely with genomic sequencing results, reinforcing its reliability as an early detection tool. New variants continue to emerge, integrating SGAF based approaches into routine laboratory workflows will remain an essential strategy for global surveillance and pandemic management.

Keywords: SARS-CoV-2, STGF, Spike gene amplification failure, Alpha, Omicron, Taqpath RT-PCR, Next generation sequencing, VOC.

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1. Introduction

Over time, viruses change and gain mutations as they replicate and infect new hosts.[1,2] Several SARS-CoV-2 lineages with various known mutations have been described by researchers since the beginning of the COVID-19 pandemic. The development of lineages with distinct behavioral traits has been noted, although it is expected that most of these mutations have no biological consequences.

Since its emergence in December 2019, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has caused over 550 million illnesses and more than 6 million fatalities.[3] To monitor and respond to the evolving threat of SARS-CoV-2, both the World Health Organization (WHO) and the U.S. Centers for Disease Control and Prevention (CDC) have established standardized criteria for the classification of viral variants. These classifications are based on multiple factors, including increased transmissibility, enhanced pathogenicity or severity of disease, and the potential to evade diagnostic detection, therapeutic efficacy, or immune protection conferred by vaccines. SARS-CoV-2 variants are categorized into three primary groups based on their genomic characteristics and potential public health

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impact: Variants of Interest (VOIs), Variants of Concern (VOCs), and Variants Under Monitoring (VUMs). This classification framework facilitates global surveillance and risk assessment of emerging lineages.[4,5] Although the S protein is usually the most impacted, each VOC has a unique array of mutations in several other genes.

The first SARS-CoV-2 Variant of Concern (VOC) was identified in the United Kingdom in late 2020, marking a significant shift in the pandemic due to its enhanced transmissibility compared to previously circulating strains.[6] The TaqPath kit for Covid-19 detection by Thermo Fisher Scientific, a extensively used kit, failed to identify the target sequence of the spike gene of SARS-CoV-2 that confirmed positive for SARS-CoV-2 RNA, which was the first indication of SARS-CoV-2 variant had emerged. The S gene (spike), the ORF gene (open reading frame), and the N gene (nucleocapsid) are the three target gene sequences of SARS-CoV-2 that can be detected using the TaqPath COVID-19 multiplex RT-PCR assay. If at least two of the three gene target sequences are found in a sample analyzed using the TaqPath RT-PCR, the test is considered positive.

In the present study, the samples which were received during January 2021 to August 2022, we have observed that the number of samples raised for the S gene was not detected, and the ORF and N genes were identified in the RT-PCR test. This Spike gene detection failure can become a representation of the evolving VOC. Several variations in this new variant were discovered by WGS of SGAF samples. These include an SGAF signature seen on the RT-PCR by TaqPath kit, which is produced by nucleotide deletions in the Spike gene that lead to the aminoacid codon 69 and 70 deletion (del 69–70). The variant was classified within the B.1.1.7 lineage according to the Phylogenetic Assignment of Named Global Outbreak Lineages (Pango) system and was later designated as the Alpha variant by the World Health Organization (WHO), in accordance with its standardized nomenclature for Variants of Concern (VOCs).[7] The TaqPath RT-PCR or additional comparable assays were employed to detect the SGAF as a stand-in for the Alpha variant's presence when its prevalence rose globally.[8] By the end of May 2021, Alpha was replaced by a new VOC, B1.617.2 or Delta, and Delta had become the most prevailing VOC by December 2021.[3] The occurrence or lack of the SGAF signature allowed samples positive for the Delta variant to be distinguished from those positive for the Alpha variant because the Delta variant did not contain the identical mutation del 69–70 in its Spike gene. In November 2021, the WHO designated Omicron as a VOC.

The B.1.1.529 by PANGO lineage is currently included in Omicron,[9] which was primarily discovered by the SALL lab in Pretoria. Other notable sub-lineages are BA.1, BA.1.1, BA.2, BA.3, BA.4, and BA.5 each exhibiting distinct mutational profiles and varying degrees of transmissibility, immune evasion, and epidemiological impact.[10] Like the Alpha variant, the spike gene del 69–70 causes the SGAF signature to be present in several Omicron sub-lineages.[11] The dynamic emergence and decline of Variants of Concern (VOCs), along with the presence or absence of the S-gene amplification failure (SGAF) signature, highlight the utility of this PCR-based marker for quick preliminary classification of circulating VOCs.

2. Materials and Methods

2.1. Sample collection and RNA isolation

550 Nasopharyngeal swab samples from COVID-19-positive samples were selected. The MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit was used on an automated KingFisher™ Flex Purification System to extract total nucleic acid. The samples collected between January 2021 to August 2022 and previous SARS-CoV-2 detected RNA elutions considered in RT-PCR using the Taqpath (reverse transcriptase) kit, which spans COVID-19 pandemic variant waves of Alpha, Delta, and Omicron in the gujarat.

2.2. RT-PCR of SARA-CoV-2 virus

The TaqPath Covid-19 RT-PCR Kit (Thermo Fisher Scientific) detects the N gene, S gene, and ORF gene. The test was performed on nasal/throat swab samples collected in VTM (Viral Transport Media) as per kit instructions (n=430). Samples were declared positive if at least two of the three gene targets were detected and the cycle threshold (Ct) value was less than 30 (n=258). (**Table 1**)

2.3. Whole genome sequencing (WGS) of SARS-CoV-2 virus

Quantification of RNA was done by Qubit HS RNA kit (TFS). A total of 258 samples were considered for cDNA synthesis using SuperScript™ VILO™ Master Mix (TFS) as per the manufacturer's instructions. Libraries were prepared following standard Ion AmpliSeq SARS-CoV-2 Research Panel protocols. The prepared libraries were loaded on Ion 540 chip, and NGS was done on Thrmofisher Iontorrent S5 plus System. FASTA files were transferred from the Torrent Suite software version 5.0.5. Alignment was performed against the Wuhan-Hu-1 reference genome using the Nextstrain database.

2.4. Statistical analysis

The analytical performance of the SGAF marker for identifying the Alpha and Omicron variants was assessed, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Cohen's kappa coefficient (κ) was interpreted using following thresholds to evaluate the level of agreement to the SGAF signature and whole genome sequencing: Standards ≤ 0 as no matching, 0.01–0.20 as none to marginal matching, 0.21–0.40 as rational matching, 0.41–0.60 as sufficient matching, 0.61–0.80 as significant matching, and 0.81–1.00 as nearly perfect matching. The sensitivity of SGAF signature detection was measured by its ability to correctly classify Alpha and

Omicron variants as true positives, whereas specificity was defined as its capacity to exclude these variants in their absence (true negatives). NPV and PPV were defined as the probability of absence (NPV) or presence (PPV) of the SGAF marker appropriately corresponded to the present or absent of the Omicron (B.1.1529, BA.1, BA.4, and BA.5) or Alpha variants. The statistical data analyses were done on GraphPad Prism version 9.5.0.

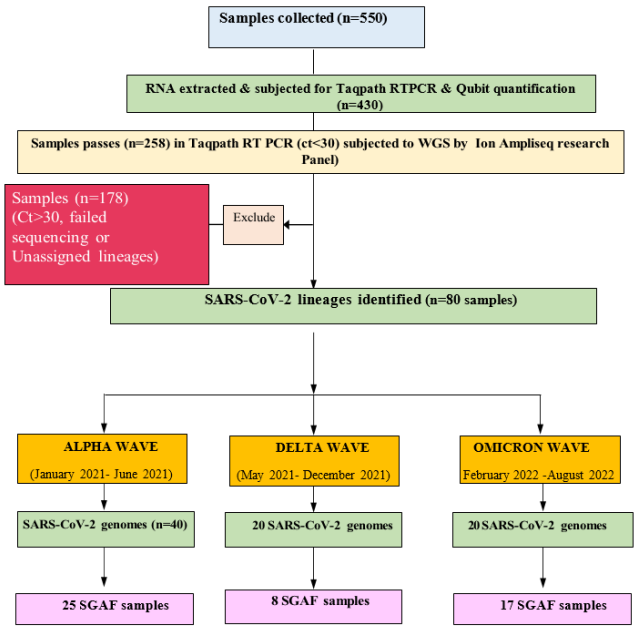

Figure 1: Graphical distribution of samples with SARS-CoV-2 wavevise genome sequences samples used in this study. WGS - Whole Genome Sequencing, SGAF- Spike Gene Amplification Failure, Ct- cycle threshold of RT PCR.

3. Results

TaqPath RT-PCR testing was conducted on a total of 550 samples as part of this study (**Figure 1**). Of these, 125 samples were processed between January 2021 and June 2021, corresponding to the Alpha variant wave in New York City. Another 130 samples were tested between Fabruary 2022 to July 2022, aligning with the Omicron wave—sublineages BA.1/BA.1.1 (January 2022– April 2022), BA.2/BA.2.12.1 (April-May 2022), and BA.4/BA.5 (July–August 2022). Between June 2021 and November 2021, the delta variant (B.1.617.2) wave occurred during which 348 samples were processed using the TaqPath assay. Overall Covid 19 detection rate by TaqPath kit was 3.0% (181/603), with 45% (82/181) of the positive samples exhibiting the SGAF signature.

Out of the SARS-CoV-2 positive samples, (n=430) with cycle threshold (Ct) values below 30 were selected for next-generation sequencing (NGS). Among these, 258 were sequenced successfully and given a specific viral lineage. SGAF signature detected by TaqPath RT-PCR demonstrated a strong concordance with NGS results for identifying Alpha and Omicron variants, showing 99.3% overall agreement and a Cohen's kappa value (κ) of 0.99, indicative of near-perfect agreement (**Table 2** and **3**). Detailed variant-wise data are provided in **Table 3**. During the Alpha variant wave, 40 genomes were sequenced, 25 of which were SGAF-positive; all 25 (62.5%) were confirmed as Alpha lineage (**Figure 2**). Additionally, eight SGAF-detected samples were identified during the Delta wave (8/216), and all were confirmed as Alpha, yielding a sensitivity of 99.7% (95% CI: 97–99.9%) and a specificity of 99.6% (95% CI: 99.2–99.8%) for Alpha variant detection using SGAF signature (**Table 4**).

Table 1: Taqpath RT PCR Processed samples with cycle threshold (ct value obtained) and no of STGF samples considered in the study (n=142). SGAF – Spike Gene Amplification Failure results of few samples listed in the table.

S.No	sample id	ORF GENE (ct)	N GENE (ct)	S GENE (ct)
1	5485	19	19	Amplification Failure (SGAF)
2	1234	19	19	Amplification Failure (SGAF)
3	5394	18	17	18
4	5382	17	18	Amplification Failure (SGAF)
5	1747	25	20	21
6	0787	19	19	Amplification Failure (SGAF)
7	0788	17	17	Amplification Failure (SGAF)
8	0772	20	20	Amplification Failure (SGAF)
9	5392	15	14	14
10	0511	17	16	Amplification Failure (SGAF)
11	0342	19	19	20
12	0340	15	14	15
13	0229	20	20	22
14	0321	20	21	Amplification Failure (SGAF)
15	0692	20	20	20
16	5393	19	19	Amplification Failure (SGAF)

17	0367	21	20	21
18	7058	21	20	21
19	7079	19	19	Amplification Failure (SGAF)
20	0295	23	23	23

Table 2: Diagnostic performance of the SGAF signature compared to NGS for detection of SARS-CoV-2 Alpha and Omicron variants

TaqPath RT-PCR (SGAF Status)	Alpha/Omicron VOC	Non-Alpha/Omicron VOC	No of Samples
SGAF Detected	80	178	258
SGAF Not Detected	2	170	172
Total	82	348	430

Table 3: Analytical performance metrics of SGAF signature relative to NGS

Performance Metric	Value (%)	95% Confidence Interval (CI)
Sensitivity	99.7	99.0–99.9
Specificity	99.1	98.4–99.5
Positive Predictive Value (PPV)	98.6	97.6–99.2
Negative Predictive Value (NPV)	99.8	99.4–99.9
Overall Agreement	99.3	–
Cohen's Kappa (κ)	0.98	–

Table 4: Performance Characteristics of the SGAF Signature in Identifying SARS-CoV-2 Variants Compared to Whole Genome Sequencing **SGAF**: S-Gene Amplification Failure as detected by TaqPath RT-PCR **WGS**: Whole Genome Sequencing **VOC**: Variant of Concern **N/A**: Not applicable (no true positive cases for those lineages to calculate sensitivity) **CI** : Confidence Interval.[17]

SARS-CoV-2 Variant (WGS Lineage)	SGAF Positive (N)	SGAF Negative (N)	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]
Alpha VOC *(B.1.1.7 and Q lineages)*	121	0	99.7 (97.3–99.9)	99.4 (97.4–99.7)
Delta VOC *(B.1.617.2 and AY lineages)*	0	190	N/A	99.0 (99.8–100.0)
Omicron VOC *(BA.1, BA.4, BA.5, B.1.1.529)*	135	2	99.4 (98.7–98.9)	99.6 (99.3–99.7)
Omicron VOC *(BA.2, BA.3)*	0	31	N/A	99.5 (97.8–99.08)
Additional SARS-CoV-2 variants	2	69	N/A	99.4 (98.9–99.7)
All Combined Variants	258	292	99.7 (99.0–99.9)	99.0 (98.4–99.5)

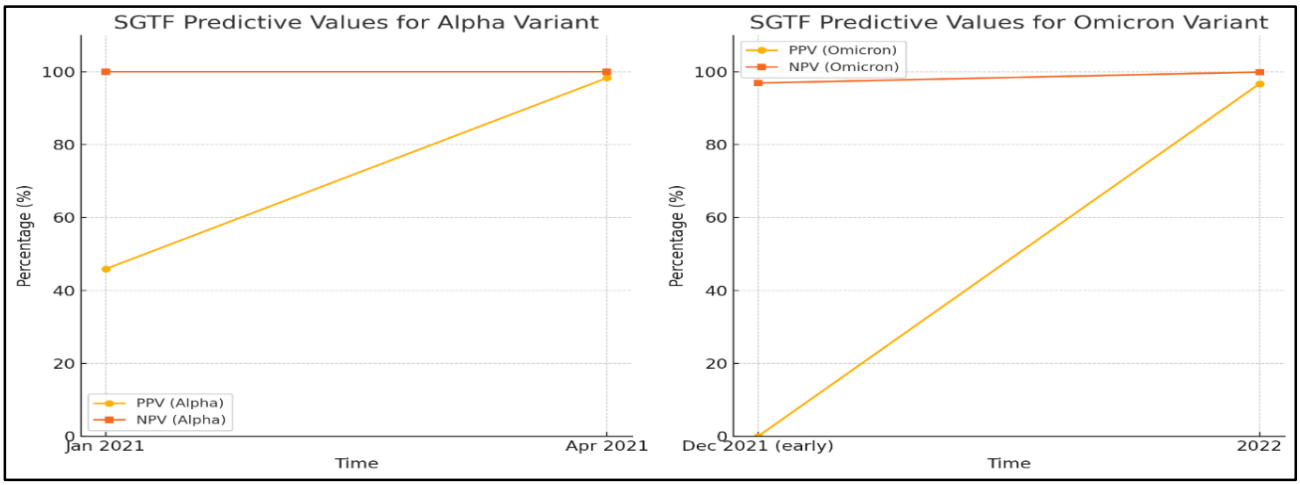

Figure 2: Alpha and Omicron variants' SGAF's Positive Predictive Value (PPV) and Negative Predictive Value (NPV) throughout time.

VOC: Variant of Concern, NGS: Next-Generation Sequencing, SGAF: Spike-Gene Amplification Failure (RT-PCR signature), PPV/NPV: Predictive values based on true positive/negative classifications, CI: Confidence Interval, κ (Kappa): Statistical measure of interrater agreement, Sensitivity: Percent of samples properly recognized as Alpha VOC or Omicron VOC (B.1.1. 529, BA.1, BA.4, BA.5) based on the presence of the SGAF detection. Specificity: Percent of sample properly recognised as NOT Alpha VOC or Omicron VOC (B.1.1. 529, BA.1, BA.4, BA.5) based on the absence of the SGAF detection.[17]

Twenty SARS-CoV-2 genomes were successfully sequenced (February 2022–August 2022) during the Omicron wave, including 17 SGAF-positive samples. Of these, 17/20 (85%) were identified as Omicron (BA.1, BA.4, BA.5, B.1.1.529) (**Figure 2**). Sequencing yielded an overall sensitivity of 99.4% (98.7–98.9%) and a specificity of 99.6% (95% CI 99.3–99.7%) for Omicron variants (B.1.1529, BA.1, BA.4, and BA.5) for two non-SGAF samples that were identified as Omicron (BA.1).

The SGAF's PPV and NPV were 83.7% and 100%, respectively, based on Gujarat State's 8%[12] collectively occurrence of the Alpha variation. With a 54% overall prevalence, the Omicron variation[13], the PPV and NPV were 99.8% and 99.5%, respectively. The test positivity rate and all sequenced cases were used to determine Gujarat's monthly prevalence of SARS-CoV-2 variants. The SGAF signature's PPV and NPV for the Alpha variant were 44.8% and 99.9% in February 2021, respectively, whereas they were 98.3% and 100% in April 2021 (**Figure 2**). Following a sharp increase in occurrence in December 2021, the Omicron variant's NPV ranged from 96.9 to 99.9%, while its PPV ranged from 0% in the initial weeks of December to 96.7 to 99.9% in 2022. Three (n=3) SGAF detected samples included the S gene deletion 69–70, which has been associated with the failure in PCR.

4. Discussion

In this study, we evaluated the TaqPath RT-PCR SGAF marker's diagnostic capability for the preliminary detection of the Alpha (B.1.1.7) and Omicron variants (B.1.1.529, BA.1, BA.4 and BA.5). For both VOCs, the sensitivity and specificity were outstanding (i.e., over 95%). The PPV increased noticeably with each variant's prevalence. The high negative predictive value (NPV) observed in our cohort indicates that, with the exception of BA.2 and BA.3 sub-lineages, samples exhibiting detectable S-gene targets by TaqPath RT-PCR kit (TFS) were indirectly represent Alpha or Omicron variants. Variant-specific RT-PCR assays have been developed based on the detection of characteristic mutations, including single-nucleotide polymorphisms (e.g., N501Y) and deletions (e.g., Δ69–70) that are unique to certain SARS-CoV-2 lineages.[13] A procedure that depends on the continuous overhauling of a variant-specific RT-PCR, be that as it may, may not be realistic for numerous research facilities and unsustainable for others due to the evolution and decrease of SARS-CoV-2 variants throughout the pandemic. Assays which have the S gene as a target gives a broadly accessible method of detection for the presence of Variants of Concern (VOCs), such as alpha or omicron, in an elementary way. This has been named the "S quality advantage." The occurrence of the SGAF marker was concordant with the Alpha variant's appearance in Toronto in one paper that focused on it, with a specificity of almost 98%, which is concordant with findings of the current study[14]. An early investigation revealed both false-positive and false-negative SGAF for the Alpha variant, which were ascribed to the analytical problems with the test (such as a false amplification signal for the S gene) and the concurrent high prevalence of other variant the del 69–70 (B.1.528)[15,16]. In this study, we also found a small number of samples that tested false positive and false negative for Omicron and Alpha variants, most likely with the similar origin. In keeping with our research, a prior South African report confirmed that the TaqPath SGAF marker had a high precision of 97.5% in recognizing the Omicron BA.1 sublineage. The study employed a genotypic assay, which targets the co-occurrence of the Δ69–70 deletion and the K417N mutation, as the gold standard method for detecting the Omicron BA.1 subvariant while excluding Alpha and Beta variants, with a subset of results verified by whole genome sequencing (WGS).[16] For optimum usage of the SGAF marker, it is critical to recognize the local occurrence of developing variants, as the deletion 69–70 exists in VOCs other than Omicron and Alpha. Therefore, the straightforward discovery of this genetic signature, which offers quick information of likely circulating variants, has a significant influence on contact investigations and patient management.

There are several limitations to our investigation. First off, our findings might not be broadly applicable to other healthcare facilities because the study was conducted at our laboratory facility and was a single-site investigation. Second, not all possible SGAF samples were included because the laboratory used several platforms for testing, therefore, not all samples were evaluated utilizing the TaqPath COVID-19 test. Third, the majority of the sequenced samples had comparatively high viral loads (Ct < 30), which may have caused bias in the data. In spite of these limitations, our study reveals a dataset assessing the diagnostic appearance of the SGAF marker across two VOCs. Notably, the FDA has not approved the TaqPath COVID-19 test, particularly for identifying SARS-CoV-2 VOCs to make treatment decisions. To inform direct patient care, laboratory professionals, physicians, and epidemiologists may find the data reported in this study helpful in providing rapid, preliminary information on circulating VOCs at the local level.

5. Conclusion

The Spike-Gene Amplification failure (SGAF) has appeared as a highly precise genomic marker for detecting specific SARS-CoV-2 variants. Its high sensitivity and specificity make it a rapid and reliable proxy for variant detection in real-world laboratory settings. The SGAF (S-Gene Amplification Failure) signature serves as a valuable molecular marker for monitoring the dissemination of SARS-CoV-2 variants harboring S-gene deletions, particularly the Alpha (B.1.1.7) and Omicron (B.1.1.529) lineages, which are linked with higher transmissibility with potential immune escape. In practical laboratory applications, SGAF testing offers several advantages over whole-genome sequencing. While sequencing remains the gold standard method for variant confirmation.

6. Data Availability

All data generated in this study are included in the manuscript.

7. Ethics Statement

Not Applicable.

8. Authors' Contribution

ND conceptualized the study, performed literature review and lab experiments. ND wrote the manuscript. CR critically reviewed and revised the manuscript. NA supervised the study experiments. All the authors read and approved the final manuscript for publication.

9. Source of Funding

None.

10. Conflict of Interest

It is declared that the authors have no conflict of interest in the publication of this article.

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