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Detection of the SARS-CoV-2 Omicron Variant in COVID-19 Patients from South Tangerang Using SNP-Probes S371L and K417N

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Abstract

The COVID-19 pandemic caused by the SARS-CoV-2 virus has posed a global challenge. Experts from various branches of science have endeavoured to find solutions to control its spread, one of which has been the quick and precise detection of the virus and its variants in patients. This study aimed to detect the presence of SARS-CoV-2, notably the rapidly spreading Omicron variant, using the spike (S)-gene target failure (SGTF) and S-gene target positive (SGTP) with the principle of the single nucleotide polymorphism (SNP)-probe test. Our descriptive experimental approach detected Omicron variants with the SNP-probe technique using samples of SARS-CoV-2 patients and controls. The probes were designed to recognize the nucleotide code of the amino acids in positions 371 and 417 of SARS-CoV-2. The existence of variants was monitored by the presence or absence of a fluorescence signal, which was translated into a sigmoidal graph using a real-time (RT)-PCR machine. One hundred and twelve samples that had tested positive for SARS-CoV-2 and the Omicron variant using a registered commercial kit showed a similar result to our in-house-developed SNP-probe 371 and 417 assays. The results of this study indicate that the SNP-probe we designed can be used in the detection of the SARS-CoV-2 Omicron variant.

Keywords: Coronavirus, COVID-19, Omicron Variant, RT-PCR, SARS-CoV-2, SGTF, SGTP, SNP-probe

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INTRODUCTION

COVID-19 caused by the SARS-CoV-2 virus has become a worldwide problem. In Indonesia, the first three cases were reported in March 2020. Cases numbers rose slowly until January 2021, then fell from February to June 2021. They increased again in July 2021, reaching almost 50,000 cases, and then from July 2021 to early 2022 to reach almost 60,000 new cases in total.[1-3]

Changes in the genome of the virus have been reported, resulting in several SARS-CoV-2 variants. The detection of SARS-CoV-2 variants is necessary to monitor and follow the development of COVID-19 cases; however, whole-genome sequencing (WGS) is both costly and time-consuming.[4,5] Based on WGS examination, the Indonesian Ministry of Health reported that the rise in case numbers during the period July to October 2021 was caused by the Delta variant of SARS-CoV-2, while the cases from January to February 2022 were caused by the Omicron variant.[6]

WGS examination can be performed in only a few laboratories in Indonesia as it requires expensive equipment and adequate infrastructure. Our institution, as one of the referral laboratories for COVID-19 diagnosis in Banten province, only conducts WGS in certain cases due to the expensive and complicated process. The results of all cases were reported either to the Indonesian Ministry of Health or the Global Initiative on Sharing All Influenza Data (GISAID) website. Therefore, it is necessary to develop a simpler method for detecting SARS-CoV-2 variants as accurately as commercial kits and that can examine many samples in a short time.

A single nucleotide polymorphism (SNP)-probe can be used as an alternative to detect variants in an organism. Through this method, we can identify different types of variants of the same species based on changes in one (or two) nucleotide bases of the target gene. Many SNP-probe methods have been developed, both with probe hybridization and hydrolysis, and these methods are useful in many applications for the identification of conditions occurring in an organism.[7-9]

In early 2020, the first SARS-CoV-2 sequencing was reported as the ancestral Wuhan sequence. Over time, more SARS-CoV-2 sequences have been reported either through GISAID or otherwise, enabling WHO to classify the class of SARS-CoV-2 variants.[10-12]

Based on our results of sequencing the SARS-CoV-2 genome and a review of the GISAID literature, we developed a screening method for the Omicron variant. Because of its ability to spread quickly, a fast and accurate detection method was needed to assist in collecting data on Omicron COVID-19 cases.

MATERIALS AND METHODS

Primer, Probe, g-Block Design

Before identifying the Omicron variant from our samples, we designed the primer, probe and g-block, specific for the spike (S)-gene targeting amino acids 371 and 417. Referring to GISAID and the next genome sequencing (NGS) results, we grouped frequently encountered or reported amino acid changes. Table 1 shows the groupings of amino acid changes in each variant, from which amino acid sequence we reversed to the nucleotide sequences. The latter was used to design the primers, probes, and g-blocks. Primers and probes were selected according to the criteria for their requirements. For SARS-CoV-2 primers targeting the S-gene, we selected areas of the nucleotide sequence unchanged for all variants, either ancestral Wuhan or the Alpha to Omicron variants. For the probes, we performed a region-specific selection of amino acids 371 and 417; both Wuhan types were used for S-gene target failure (SGTF) while Omicron was used for S-gene target positive (SGTP).

We used g-blocks as positive or negative controls for both Wuhan and Omicron, SGTF or SGTP, respectively. We also used NGS-positive samples for comparison. In SGTF, the probe attachment to the Wuhan g-block or the Wuhan-NGS variant control sample served as a negative control, while neither attachment to the Omicron g-block nor the Omicron-NGS control sample served as a positive control, and vice versa with SGTP. The primer-probe sequences are listed in Table 1 and the g-block sequences are shown in Table 2.

Table 1. Primer and Probe Sequences

Primer 371	Forward	5'- TGG AAC AGG AAG AGA ATC AGC A -3'
	Reverse	5'- AGT AGG AGA CAC TCC ATA ACA CT -3'
Probe SGTF 371	Probe 1	5'- TCC GCA TCA TTT TCC ACT TT -3'
Probe SGTP 371	Probe 1	5'- CTC GCA CCA TTT TTC ACT TT -3'
Primer 417	Forward	5'- TCA GAC AAA TCG CTC CAG GG -3'
	Reverse	5'- CAA GCT ATA ACG CAG CCT GT -3'
Probe SGTF 417	Probe 2	5'- AAG ATT GCT GAT TAT AAT TA -3'
Probe SGTP 417	Probe 2	5'- AAT ATT GCT GAT TAT AAT TA -3'

Table 2. g-Block Sequences for Wuhan and Omicron

g-BlockType/ Variant	Nucleotide Sequence
Wuhan	CGAAGACCCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTTGT AATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAAGTGAGTTCAGAGTTTAT TCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCCTTTTCTTATGGACCTTGAAGGAAAACAGGGTA ATTTCAAAAATCTTAGGGAATTTGTGTTTAAGAATATTGATGGTTATTTTAAAATATATTCTAAGCACACGCCTA TTAATTTAGTGCGTGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTTGCCAATAGGTATTAAC ATCACTAGGTTTCAAACTTTACTTGCTTTACATAGAAGTTATTTGACTCCTGGTGATTCTTCTTCAGGTTGGAC AGCTGGTGCTGCAGCTTATTATGTGGGTTATCTTCAACCTAGGACTTTTCTATTAAAATATAATGAAAATGGAA CCATTACAGATGCTGTAGACTGTGCACTTGACCCTCTCTCAGAAACAAAGTGTACGTTGAAATCCTTCACTGTA GAAAAAGGAATCTATCAAACTTCTAACTTTAGAGTCCAACCAACAGAATCTATTGTTAGATTTCCTAATATTAC AAACTTGTGCCCTTTTGGTGAAGTTTTTAACGCCACCAGATTTGCATCTGTTTATGCTTGGAACAGGAAGAG AATCAGCAACTGTGTTGCTGATTATTCTGTCCTATATAATTCCGCATCATTTTCCACTTTTAAGTGTTATGGAGT GTCTCCTACTAAATTAAATGATCTCTGCTTTACTAATGTCTATGCAGATTCATTTGTAATTAGAGGTGATGAAGT CAGACAAATCGCTCCAGGGCAAACTGGAAAGATTGCTGATTATAATTATAAATTACCAGATGATTTTACAGGC TGCGTTATAGCTTGGAATTCTAACAATCTTGATTCTAAGGTTGGTGGTAATTATAATTACCTGTATAGATTGTTTA GGAAGTCTAATCTCAAACCTTTTGAGAGAGATATTTCAACTGAAATCTATCAGGCCGGTAGCACACCTTGTAA TGGTGTTGAAGGTTTTAATTGTTACTTTCCTTTACAATCATATGGTTTCCAACCCACTAATGGTGTTGGTTACC AACCATACAGAGTAGTAGTACTTT
Omicron	CGAAGACCCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTTGTA ATGATCCATTTTTGGATGTTTATCACCACAAAAACAACAAAAGTTGGATGGAAAGTGGAGTTTATTCTAGTGC GAATAATTGCACTTTTGAATATGTCTCTCAGCCTTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTTCAAA AATCTTAGGGAATTTGTGTTTAAGAATATTGATGGTTATTTTAAAATATATTCTAAGCACACGCCTATTAATTTA GTGCGTGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTTGCCAATAGGTATTAACATCACTAG GTTTCAAACTTTACTTGCTTTACATACAAGTTATTTGACTCCTGGTGATTCTTCTTCAGGTTGGACAGCTGGTG CTGCAGCTTATTATGTGGGTTATCTTCAACCTAGGACTTTTCTATTAAAATATAATGAAAATGGAACCATTACAG ATGCTGTAGACTGTGCACTTGACCCTCTCTCAGAAACAAAGTGTACGTTGAAATCCTTCACTGTAGAAAAAGG AATCTATCAAACTTCTAACTTTAGAGTCCAACCAACAGAATCTATTGTTAGATTTCCTAATATTACAAACTTGTG CCCTTTTGGTGAAGTTTTTAACGCCACCAGATTTGCATCTGTTTATGCTTGGAACAGGAAGAGAATCAGCAA CTGTGTTGCTGATTATTCTGTCCTATATAATTCCGCATCATTTTCCACTTTTAAGTGTTATGGAGTGTCTCCTACT AAATTAAATGATCTCTGCTTTACTAATGTCTATGCAGATTCATTTGTAATTAGAGGTGATGAAGTCAGACAAAT CGCTCCAGGGCAAACTGGAAATATTGCTGATTATAATTATAAATTACCAGATGATTTTACAGGCTGCGTTATAG CTTGGAATTCTAACAATCTTGATTCTAAGGTTGGTGGTAATTATAATTACCTGTATAGATTGTTTAGGAAGTCTA ATCTCAAACCTTTTGAGAGAGATATTTCAACTGAAATCTATCAGGCCGGTAGCACACCTTGTAATGGTGTTGA AGGTTTTAATTGTTACTTTCCTTTACAATCATATGGTTTCCAACCCACTAATGGTGTTGGTTACCAACCATACAGA GTAGTAGTACTTT

Table 3. The Position of the Amino Acid Changes in the Spike (S) Gene

Variant type	Amino acid position in S gene target			
	1 – 300	301 – 600	601 – 900	901 - 1200
Alpha B.1.1.7	Del69-70HV; del144Y	N501Y; A570D	D614G; P681H; T716I	S982A; D1118H
Bata B.1.351	L18F; D80A; D215G; del242-244; R246I	K417N; E484K; N501Y	D614G; A701V	-
Gamma P.1	L18F; T20N; P26S; D138Y; R190S	K417T; E484K; N501Y	D614G; H655Y	T1027I
Delta B.1.617.2	T19R; V70F; T95I; G142D; del156; del157; R158G; A222V; W258L	K417N; L452R; T478K	D614G; H681R	D950N
Omicron B.1.1.529	A67V;del69/70HV; T95I; delG142; delV143; delY144; Y145D; delN211; L212I	G339D; S371L; S373P; S375F; K417N; N440K; G446S; S477N; T478K; E484A; Q493R; G496S; Q498R; T547K	D614G; H655Y; N679K; P681H; N764K; D796Y; N856K	Q954H:N969K

Note to Table 3: The position of amino acid changes in the spike (S) gene was used as reference for the design of primers and probes

qPCR Analysis

A total of 112 samples from COVID-19 patients who returned a positive SARS-CoV-2 test using the Standard M-nCoV M-NCOV-01 (BioSensor, Korea) were tested for variant confirmation with the SNPsig®-SARS-CoV-2-EscapePLEX (Primerdesign, UK) and VarScreen-RXReady® mBioCoV-19 (BioFarma, Indonesia) kits. Samples that were positive for Omicron were further investigated by SNP-probe examination, with the target S-gene at amino acid positions 371 and 417. We used a LightCycler 480 II (Roche-Germany) with the following program: activation of the reverse transcriptase at 55°C for 10 minutes; one deactivation cycle at 95°C for 2 min; PCR: one denaturation cycle at 95°C for 10 seconds; annealing at 55°C and reading of the dye signal for one minute, PCR program 45 cycles. Each examination was conducted in triplicate and the results were confirmed by measurements on three different days. The samples tested had Ct values from COVID-19 RT-PCR examination of between 20 and 33. For confirmation, three samples that were positive for the Omicron variant were examined for the whole genome using Oxford Nanopore Technology (ONT)-UK.

RESULTS

Number of Cases in South Tangerang

Figure 1 shows the timeline of the COVID-19 cases previously tested from June 2020 to March 2022 in our laboratory. More than 15,000 samples were tested and 6,856 were positive for COVID-19. We obtained samples of COVID-19 patients from hospitals in the region of the Faculty of Medicine, State Islamic University Syarif Hidayatullah Jakarta, namely the South Tangerang area.

Design of Primer and SGTF-SGTP Probe

To detect the SARS-CoV-2 Omicron variant, we designed a primer and specific probe targeting the S-gene. Table 3 shows the amino acid position of SARS-CoV-2 in the S-gene.

After optimisation, we used the primer and probe to identify the Omicron variant in our samples. We used only samples that had already tested positive for Omicron using commercial kits (SNPsig®-SARS-CoV-2-EscapePLEX and VarScreen-RXReady mBioCoV-19).

Case numbers
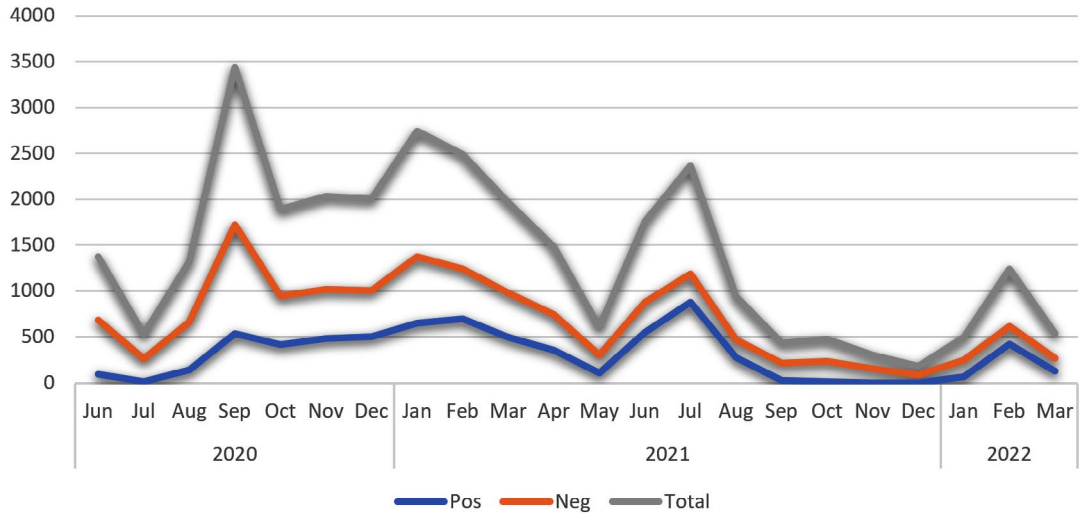
Figure 1. The sample numbers of COVID-19 cases in the Laboratory of Microbiology Research, Faculty of Medicine, State Islamic University from June 2020 to March 2022. The grey line is the total number of incoming samples for the PCR test. The orange line denotes the negative results, and the blue line shows the positive results after PCR
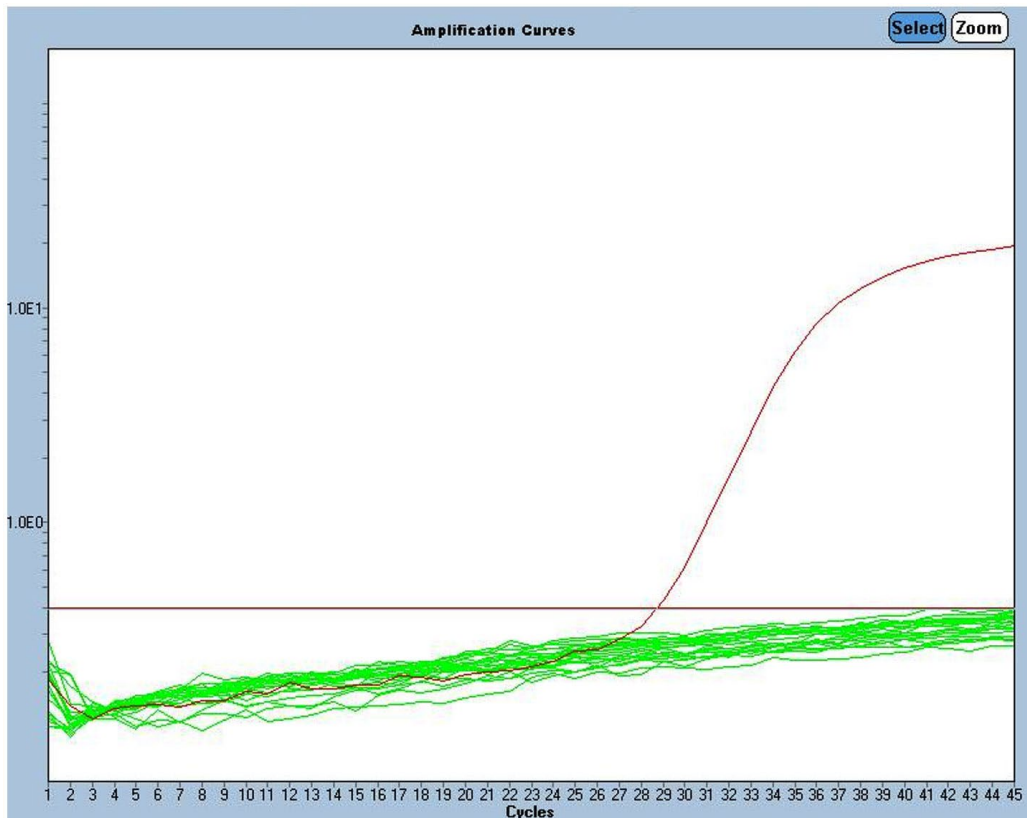
Figure 2. S-gene target failure (SGTF) probe 371; the green lines denote non-amplification; the red line is the amplification graph of g-block Wuhan

Detection of SARS-CoV-2 by Probes 371 and 417 and Confirmed With Established Kit

The 112 samples that tested positive for SARS-CoV-2 in both the RT-PCR test and the variant examination using SNPsig®-SARS-CoV-2 and VarScreen-RXReady were used for the SGTF and SGTP examination of the Omicron variant by SNP-probes with target amino acids S371L and K417N. Figures 2 and 3 show the amplification process for the detection of the Omicron variant using our primers and probes.

Figures 2 and 3 show the SGTF and SGTP examinations. In SGTF, only g-block or synthetic nucleotide Wuhan would amplify with the SGTF probe, whereas the Omicron samples would not. The SGTF probe only binds with the Wuhan nucleotide sequence; if a nucleotide in this sequence is changed, the probe will not bind. No amplification signal will appear if the probe does not bind to the sample. The same principle concerning the probe applies in both SGTP and SGTF; however, the probe will only bind to samples that have the nucleotide sequence of the Omicron variant. Thus, the principle of the SGTP probe is the opposite of that for SGTF.

The selection of target amino acid position 371 was based on the WGS SARS-CoV-2 sequencing of the Omicron variant compared to other variants such as Alpha, Beta, Gamma, My and Delta. In the WGS analysis, the S-gene amino acid target 371 (at the nucleotide position 21,000's) is in an area of change found exclusively in Omicron and thus not in other variants. As for target amino acid 417, this change is found in Omicron, Delta and Beta.

For the position of the S371L gene, we developed the SGTF and SGTP methods, where SGTF will only be positive for amplification results in the form of Ct and sigmoid curves for non-Omicron variants, while SGTP will only be
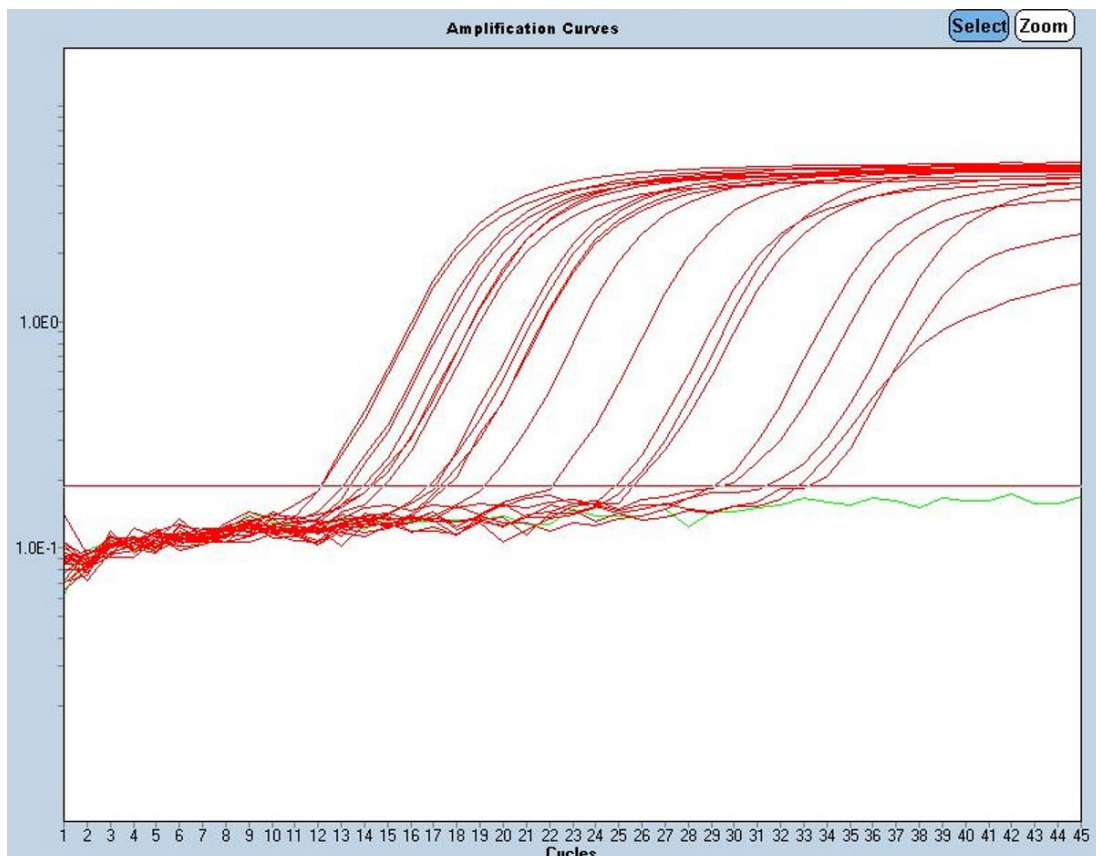

Figure 3. S-gene target positive (SGTP) probe 371; the red lines depict the amplification graphs; the green line denotes non-amplification of g-block Wuhan

Table 4. SGTF and SGTP 371/417 confirmation with established kits of SARS-CoV-2 variants

No.	Sample	SGFT Probe		SGTP Probe		SNPsig Escape PLEX SARS-CoV-2	VarScreen Rx Ready mBioCoV-19+	Conclusion
		371L FAM	417N HEX	371 FAM	417N FAM			
1.	dH$_2$O	-	-	-	-	-	-	Negative
2.	g-Block Wuhan (Positive)	+	+	-	-	Not test	Not test	COVID-19 Wuhan
3.	g-Block Omi and Delta (Positive)	-	-	+	+	Not test	Not test	COVID-19 Omi/ Delta
4.	Positive Control for Prime/ Biofarma	Not test	Not test	Not test	Not test	+	+	COVID-19 Omi
5.	14354	-	-	+	+	+	+	COVID-19 Omi
6.	14357	-	-	+	+	+	+	COVID-19 Omi
7.	14291	+	-	-	+	+	+	COVID-19 Delta
8.	11440	+	+	-	-	-	-	COVID-19 Wuhan
9.	10922	+	+	-	-	-	-	COVID-19 Wuhan
10.	14280	+	+	-	-	-	-	COVID-19 Wuhan
11.	12165	+	-	-	+	+	+	COVID-19 Delta
12.	14334	-	-	+	+	+	+	COVID-19 Omi
13.	14335	-	-	+	+	+	+	COVID-19 Omi
14.	14336	-	-	+	+	+	+	COVID-19 Omi
15.	14355	-	-	+	+	+	+	COVID-19 Omi
16.	14311	-	-	+	+	+	+	COVID-19 Omi
17.	14291	+	+	-	-	-	-	COVID-19 Wuhan
18.	14306	-	-	+	+	+	+	COVID-19 WGS OMI
19.	14332	-	-	+	+	+	+	COVID-19 Omi
20.	14333	-	-	+	+	+	+	COVID-19 Omi
21.	14331	-	-	+	+	+	-	COVID-19 WGS OMI
22.	14397	-	-	+	+	+	+	COVID-19 Omi
23.	14366	-	-	+	+	+	+	COVID-19 Omi
24.	14384	-	-	+	+	+	+	COVID-19 Omi
25.	14385	-	-	+	+	+	+	COVID-19 Omi
26.	14389	-	-	+	+	+	+	COVID-19 Omi
27.	14391	-	-	+	+	+	+	COVID-19 Omi
28.	14392	-	-	+	+	+	+	COVID-19 Omi
29.	14393	-	-	+	+	+	+	COVID-19 Omi
30.	14394	+	+	-	-	-	-	COVID-19 Wuhan
31.	14395	+	+	-	-	-	-	COVID-19 Wuhan
32.	14396	+	+	-	-	-	-	COVID-19 Wuhan
33.	14398	+	+	-	-	-	-	COVID-19 Wuhan
34.	14399	+	+	-	-	-	-	COVID-19 Wuhan
35.	14400	+	+	-	-	-	-	COVID-19 Wuhan
36.	14419	-	-	+	+	+	+	COVID-19 Omi
37.	14420	-	-	+	+	+	+	COVID-19 Omi
38.	14421	-	-	+	+	+	+	COVID-19 Omi
39.	14422	-	-	+	+	+	+	COVID-19 Omi
40.	14423	-	-	+	+	+	+	COVID-19 Omi
41.	14424	-	-	+	+	+	+	COVID-19 Omi

Table 4. Cont...

No.	Sample	SGFT Probe		SGTP Probe		SNPsig Escape PLEX SARS-CoV-2	VarScreen Rx Ready mBioCoV-19+	Conclusion
		371L FAM	417N HEX	371 FAM	417N FAM			
42.	14426	-	-	+	+	+	+	COVID-19 Omi
43.	14427	-	-	+	+	+	+	COVID-19 Omi
44.	14428	-	-	+	+	+	+	COVID-19 Omi
45.	14429	-	-	+	+	+	+	COVID-19 Omi
46.	14438	+	+	-	-	-	-	COVID-19 Wuhan
47.	10061	+	-	-	+	+	+	COVID-19Delta
48.	10922	+	+	-	-	-	-	COVID-19 Wuhan
49.	12165	+	+	-	-	-	-	COVID-19 Wuhan
50.	13597	+	-	-	+	+	+	COVID-19Delta
51.	13759	+	+	-	-	-	-	COVID-19 Wuhan
52.	10058	+	-	-	+	+	+	COVID-19Delta
53.	14764	-	-	+	+	+	+	COVID-19 Omi
54.	14931	-	-	+	+	+	+	COVID-19 Omi
55.	14820	-	-	+	+	+	+	COVID-19 Omi
56.	14747	+	+	-	-	-	-	COVID-19 Wuhan
57.	14927	+	+	-	-	-	-	COVID-19 Wuhan
58.	14819	-	-	-	-	-	-	COVID-19 Wuhan
59.	14680	-	-	+	+	+	+	COVID-19 Omi
60.	14697	-	-	+	+	+	+	COVID-19 Omi
61.	14699	-	-	+	+	+	+	COVID-19 Omi
62.	14712	-	-	+	+	+	+	COVID-19 Omi
63.	14744	-	-	+	+	+	+	COVID-19 Omi
64.	14747	-	-	+	+	+	+	COVID-19 Omi
65.	14748	-	-	+	+	+	+	COVID-19 Omi
66.	14759	-	-	+	+	+	+	COVID-19 Omi
67.	14764	-	-	+	+	+	+	COVID-19 Omi
68.	14779	-	-	+	+	+	+	COVID-19 Omi
69.	14780	-	-	+	+	+	+	COVID-19 Omi
70.	14781	-	-	+	+	+	+	COVID-19 Omi
71.	14782	-	-	+	+	+	+	COVID-19 Omi
72.	14783	-	-	+	+	+	+	COVID-19 Omi
73.	14729	-	-	+	+	+	+	COVID-19 Omi
74.	14638	-	-	+	+	+	+	COVID-19 Omi
75.	14656	-	-	+	+	+	+	COVID-19 Omi
76.	14661	-	-	+	+	+	+	COVID-19 Omi
77.	14662	-	-	+	+	+	+	COVID-19 Omi
78.	14663	-	-	+	+	+	+	COVID-19 Omi
79.	14664	-	-	+	+	+	+	COVID-19 Omi
80.	14665	-	-	+	+	+	+	COVID-19 Omi
81.	14666	-	-	+	+	+	+	COVID-19 Omi
82.	14677	-	-	+	+	+	+	COVID-19 Omi
83.	14678	-	-	+	+	+	+	COVID-19 Omi
84.	14681	-	-	+	+	+	+	COVID-19 Omi
85.	14682	-	-	+	+	+	+	COVID-19 Omi
86.	14683	-	-	+	+	+	+	COVID-19 Omi
87.	14594	-	-	+	+	+	+	COVID-19 Omi
88.	14498	-	-	+	+	+	+	COVID-19 Omi
89.	14526	-	-	+	+	+	+	COVID-19 Omi

Table 4. Cont...

No.	Sample	SGFT Probe		SGTP Probe		SNPsig Escape PLEX SARS-CoV-2	VarScreen Rx Ready mBioCoV-19+	Conclusion
		371L FAM	417N HEX	371 FAM	417N FAM			
90.	14527	-	-	+	+	+	+	COVID-19 Omi
91.	14552	-	-	+	+	+	+	COVID-19 Omi
92.	14553	-	-	+	+	+	+	COVID-19 Omi
93.	14509	-	-	+	+	+	+	COVID-19 Omi
94.	14510	-	-	+	+	+	+	COVID-19 Omi
95.	14511	-	-	+	+	+	+	COVID-19 Omi
96.	14512	-	-	+	+	+	+	COVID-19 Omi
97.	14513	-	-	+	+	+	+	COVID-19 Omi
98.	14514	-	-	+	+	+	+	COVID-19 Omi
99.	14515	-	-	+	+	+	+	COVID-19 Omi
100.	14486	-	-	+	+	+	+	COVID-19 Omi
101.	14496	-	-	+	+	+	+	COVID-19 Omi
102.	14497	-	-	+	+	+	+	COVID-19 Omi
103.	14542	-	-	+	+	+	+	COVID-19 Omi
104.	14529	-	-	+	+	+	+	COVID-19 Omi
105.	14554	-	-	+	+	+	+	COVID-19 Omi
106.	14555	-	-	+	+	+	+	COVID-19 Omi
107.	14556	-	-	+	+	+	+	COVID-19 Omi
108.	14557	-	-	+	+	+	+	COVID-19 Omi
109.	14478	-	-	+	+	+	+	COVID-19 Omi
110.	14487	-	-	+	+	+	+	COVID-19 Omi
111.	14596	-	-	+	+	+	+	COVID-19 Omi
112.	14542	-	-	+	+	+	+	COVID-19 Omi

positive for amplification results in the form of Ct and sigmoid curves for Omicron variants. As for K417N, the method we developed will only detect the Omicron, Delta and Beta variants.

Table 4 shows the results using our probes and confirmation with the two commercial SARS-CoV-2 variant kits. Here, our method returned similar results to those of the commercial kits and (to the extent it was conducted) the WGS examination of our samples.

DISCUSSION

Significant findings of our study are : (1). Through the g-block or nucleotide fragment as a positive control, the two types of probes for each target amino acid fit specifically with the ancestral Wuhan or the Omicron variant. These specific results confirmed the correctness and success of the fluorescence signal; (2). Here, our diagnostic method using probe 371 and 417 gave similar results to those of the commercial kits and (to the

extent it was conducted) the WGS examination of our samples.

Number of Cases in South Tangerang

Since the first report of SARS-CoV-2 in 2019, there have been many accounts regarding the characteristics of the virus. However, since the virus mutates very easily and changes its properties, there is unlikely to be an end to the discussion around SARS-CoV-2 and its variants.[13,14] The most recently reported variant, in late 2021, was Omicron, which can spread rapidly. However, reports stating an increase in severity and mortality in adults due to this mutation compared to the previous Delta variant have been rare.[13-20]

In December 2021, when cases of the SARS-CoV-2 Omicron variant increased worldwide, the Indonesian Ministry of Health instructed all government referral laboratories to examine this mutation, especially using WGS examination.[21,22] However, not all referral laboratories have WGS instruments and sufficient financing for such

expensive analysis, so referral laboratories were authorised to test variants using SNP or SGTF kits. Unfortunately, not every RT-PCR machine in the laboratories could be used for variant detection using the commercial test kits provided by the Ministry of Health; the kits could only be used with certain types of RT machines. Therefore, we developed a method for the detection of Omicron variants that can be used in all RT-PCR machines.

In general, the high-cost process of the genome sequencing method is used to trace SARS-CoV-2 variants. Currently, the development of PCR techniques is increasingly rapid and makes it easier to track a nucleotide change in the gene of living organisms. Several conventional PCR methods can be used to track changes in one or more nucleotides, such as restriction fragment length polymorphism (RFLP)-PCR, specific primer-PCR, SNP-PCR and so on, which generally require agarose electrophoresis for detection. These techniques are cheaper but require more time and work, meaning they are very time-consuming if more than 10 samples have to be examined. Other, non-conventional PCR techniques may be suitable, such as RT-PCR, quantitative (q) PCR or digital PCR.[23-29]

The principle of these techniques is the same as in conventional PCR; to amplify a DNA fragment. In qPCR, the thermocycler has a sensor to detect a fluorescence signal. In addition to a primer, we include a probe, an internal primer with a fluorescence dye or a reagent mix containing the fluorescence solution (such as Sbyr). Thus, we can observe the amplification directly and "real-time in-process" by applying non-conventional PCR methods such as SNP-probe PCR, Rhamp-SNP PCR, Sbyr-PCR and so on.[28,29]

Design of Primer and SGTF-SGTP Probe

The SGTF and SGTP examination was based on the identification of SNP or double nucleotide polymorphism (DNP) methods.[29,30] The position of the nucleotide change can be in the coding, non-coding or intergenic regions.[13,28-30] We developed a probe that recognises the amino acid leucine at position 371 in Omicron instead of serine in the ancestral Wuhan variant. As such, the probe will attach to the Omicron variant that has changed from 371 serine to leucine, but there will be no attachment to the ancestral variant or

other variants without such substitution. Likewise, the test probe for K417N, which involves the substitution of lysine 417 to asparagine, has the same principle as for S371L, in both SGTF and SGTP.

In our experiments, we designed two types of probes for each target amino acid, namely the ancestral Wuhan and the Omicron variant. With the g-block or nucleotide fragment as a positive control, they fit each type of probe. The existence of two probe models and g-blocks to target the SARS-CoV-2 S-gene further confirmed the correctness and success of the fluorescence signal. For detection, we used carboxyfluorescein (FAM) in a single-plex inspection reaction or a single test. The reason for choosing this fluorescence dye is that FAM sensors can be found in all RT-PCR cyclers. Thus, even in laboratories equipped with old-type RT-PCR instruments and limited signal channels, the method we developed can be considered for detecting Omicron variants.

Detection of SARS-CoV-2 by Probes 371 and 417 and Confirmed With Established Kit

Our SGTF and SGTP results were all confirmed by the same results obtained via tests using SNPsig and VarScreen reagents (Table 4). This indicates that the probes we tested can be applied for trace examinations of SARS-CoV-2 Omicron variants. In addition to using an existing kit, we confirmed the results using Oxford Nano Technology genome sequencing, demonstrating success in probe testing. Therefore, our design probe can be used not only in Indonesia but also globally. The limitation of our study derived from the patient data (hospital records) as we could only obtain swab samples with numbers and no information regarding the condition of the patients. Our laboratory is not a hospital laboratory but a medical faculty research laboratory that is seconded for COVID-19 examination in the South Tangerang area. Thus, we do not hold patient data. It would have been preferable for the hospital that sent the sample to have also provided complete data on the patient's status.

CONCLUSION AND FUTURE PROSPECTIVES

In conclusion, our research successfully developed an SNP identification method for SARS-CoV-2 variants using specific probes for SGTF/SGTP

S371L and K417N. Our method can be used for the detection of SARS-CoV-2 Omicron variants.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

CA conceptualized the study, applied methodology and performed validation. CA, EAS, LAH, ZH, S, DFR, AL, FE, EW, FRS, SK, MAAF and DRN performed analysis. CA and HJF wrote the original draft. HJF, EAS, LAH, ZH, S, DFR, AL, FE, EW, FRS, SK, MAAF and DRN reviewed and revised the manuscript. All authors read and approved the final manuscript for publication.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Ethics Committee, Faculty of Medicine UIN Syarif Hidayatullah Jakarta (No. B-005/F12/ KPK/ TL.00/02/2021) .

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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