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Utilization of *in silico*-designed primers for SARS-CoV-2 Molecular Surveillance using Direct PCR Product Sequencing Surveillance (DPPSS) Method

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ABSTRACT

Background. The COVID-19 pandemic caused by SARS-CoV-2 significantly strained healthcare systems in the Philippines, highlighting the critical importance of reliable molecular diagnostics and genomic surveillance. Although vaccination efforts and public health measures mitigated disease impact, the continued emergence of viral variants underscores the need for sustainable local surveillance strategies. Strengthening in-country capacity through the development of *in silico*–designed primers and cost-effective sequencing approaches can enhance rapid variant detection and improve preparedness for future emerging infectious diseases.

Objective. This paper offers a method in detecting the SARS-CoV-2 virus and its variants. A direct PCR product sequencing surveillance or DPPSS offers a new possibility of detecting emerging disease by using PCR products and using it as templates in determining the base sequence.

Methodology. A total of 20 random positive samples for SARS-CoV-2 from March 2022 sample pool in Metro Manila, Philippines was used in this study. The RNA was extracted using Purelink™ RNA Mini Kit, quantified with NanoDrop, and subjected to one-step RT-PCR. An in-house designed *in silico* primers were used in this study by using thermodynamic parameters to optimize specificity and amplification efficiency, considering GC contents, balanced Tm, minimal secondary structures and cross-dimers and *in silico* validation via Basic Local Alignment Search Tool (BLAST) against reference databases.

Amplicons were analyzed through gel electrophoresis, sequenced, and analyzed using BioEdit software. A nucleotide BLAST search identified COVID-19 variants, confirmed using Cov-Lineages website.

Results. *In silico* designed primers (S1, S2, E/M, Orf1ab) collectively exhibited 100% sensitivity in detecting SARS-CoV-2 in nasopharyngeal swab samples. Individual primer sensitivities varied, with Orf1ab at 58.82% and E/M at 90.91%. Our analysis revealed the prevalence of Omicron sublineage BA.2 in the Philippines, aligning with local data showing more BA.2 cases than the global predominance of BA.1.

Conclusion. Combined *in silico* primers (S1, S2, E/M, ORF1ab) accurately detect SARS-CoV-2 and its variants. This method provides a valuable diagnostic and surveillance tool for public health management.

Key words: COVID-19, virus variants, PCR, sequencing, molecular diagnostic techniques

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INTRODUCTION

In early 2020, the world faced a novel public health crisis caused by SARS-CoV-2, the etiologic agent of Coronavirus 2019 (COVID-19). The virus primarily affects the respiratory system, causing symptoms ranging from mild upper respiratory illness to severe pneumonia.[1,2] As of January 2026, the World Health Organization (WHO) reported 779,102,516 confirmed cases globally,[3] while the Philippine Department of Health documented 4,140,383 cases nationwide.[4] Multiple variants have since emerged, including Alpha, Beta, Gamma, Delta, Epsilon, Eta, Iota, Kappa, Zeta, Mu, and Omicron, with Omicron classified as a Variant of Concern and others as Variants Being Monitored.[1,2]

The pandemic highlighted the critical role of laboratory diagnostics. Nucleic acid amplification tests, particularly reverse transcription- polymerase chain reaction (RT-PCR), remain the gold standard for COVID-19 diagnosis due to their high sensitivity and specificity. However, limitations such as reduced detection in low viral load samples and resource-intensive requirements restrict widespread implementation in low- and middle-income countries.[5,6] Rapid antigen and antibody-based assays support surveillance but require confirmatory testing due to lower sensitivity. Meanwhile, genomic surveillance through next-generation and whole-genome sequencing is essential for variant characterization, though high costs limit routine clinical use.[5]

Although the WHO declared the end of COVID-19 as a global health emergency in May 2023,[7] continued viral evolution underscores the need for sustainable and cost-effective molecular surveillance strategies. SARS-CoV-2 continues to acquire mutations that influence transmissibility, immune escape, and therapeutic effectiveness. Strengthening local capacity for variant detection and preparedness remains imperative.

This study introduces Direct PCR Product Sequencing Surveillance (DPPSS) as a low-cost alternative for molecular surveillance. Direct sequencing of PCR-amplified products enables rapid base sequence determination with minimal processing errors,[8] offering a practical approach for resource-limited settings such as the Philippines. Primer design targeted key genomic regions of SARS-CoV-2, including the spike (S), envelope (E), membrane (M), and open reading frame (ORF) regions.[9-11] The S primer targets the spike protein gene associated with viral entry; the EM primer aligns with envelope and membrane regions critical for viral assembly; and the O primer spans open reading frames to provide broader genomic coverage.[12-14] These targeted regions support effective and efficient variant monitoring using the DPPSS method.

As we utilize PCR surveillance sequencing as a cornerstone in tracking and understanding the evolution of SARS-CoV-2, the chosen primers enhance the specificity and sensitivity of the assay, ensuring accurate detection and surveillance. The strategic placement of primers allows for targeted amplification of regions crucial for both diagnostic and epidemiological purposes.

This paper aims to present an evaluation *in silico*-designed primers using the DPPSS method being developed for the detection of SARS-CoV-2 variants among RNA samples extracted from SARS-CoV-2 positive nasopharyngeal swab specimens. Likewise, this paper will identify the sensitivity of the designed primers to detect the SARS-CoV-2 virus, determine the type and frequency of SARS-CoV-2 variants in the positive specimens, and correlate the relationship of the detected variants with the clinical and epidemiological data during the time of collection.

METHODOLOGY

Sampling

A total of 20 random nasopharyngeal/ oropharyngeal positive samples for SARS-CoV-2 after RT-PCR testing were taken from March 2022 sample pool in Metro Manila, Philippines.

RNA extraction

The viral RNA of the samples was extracted using the PureLink™ Viral RNA Mini Kit (Invitrogen®) by following the manufacturer's protocol. Essentially, proteinase K and a lysis buffer were used to open cells and obtain RNA. Following this, RNA binding was performed using spin-columns. Wash buffers were used for the RNA washing step and lastly, the RNA was eluted through centrifugation. The eluted RNA was stored in -20°C.

Nucleic acid quantification

The RNA extracts were quantified using the Thermo Scientific NanoDrop™ 1000 Spectrophotometer. First, the device was cleaned using sterile distilled water and bleach in an alternate manner for 2 mins each. Following this step, "blank" measurements were made by dispensing 1 μL of buffer onto the lower optical surface. Upon completing the cleaning and blank reading steps, quantification of the samples was performed by loading 1 μL of the sample onto the device. To accurately assess sample quality, 260/280 or 260/230 ratios was analyzed in combination with overall spectral quality. Pure nucleic acids typically yield a 260/280 ratio of ~1.8 and a 260/280 ratio of ~2.0 for DNA and RNA, respectively.

DPPSS primer

In silico–designed oligonucleotide primer sets targeting the ORF1ab, S1, S2, E, and M genomic regions of SARS-CoV-2 (See Table 1). The table summarizes the target gene, primer designation (forward and reverse), nucleotide sequence (5′–3′), predicted amplicon length (bp), GC content (%), and calculated melting temperature (Tm, °C). Primer design was performed using thermodynamic parameters to optimize specificity and amplification efficiency, with selection criteria including appropriate GC content, balanced Tm between primer pairs, absence of significant secondary structures (hairpins and self-dimers), minimal cross-dimer formation, and *in silico* specificity verification through Basic Local Alignment Search Tool (BLAST) analysis against reference genomic databases. It was then sent for synthesis to Macrogen, Inc. (South Korea).

One step RT-PCR

The one-step RT-PCR was performed using the SuperScript™ III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen®, USA). Following the manufacturer's protocol, each 10 μL reaction contained 2 μL of 2x Reaction buffer, 0.4 μL DNTPs, 0.6 μL of each forward and reverse primers, 0.4 μL Taq polymerase enzyme, 1 μL RNA template, and 5 μL distilled, nuclease-free water. The touchdown amplification was carried out using the T100™ thermal cycler (Bio-Rad, Japan). The thermal cycling conditions consisted of 30 mins at 50°C for reverse transcription, 15 mins at 95°C for Taq polymerase activation, 10 cycles of touchdown PCR set at 94°C for 1 min denaturation, 55°C for 1 min annealing, and 72°C for 1 min extension. After this, the final PCR profile is set at 20 cycles consisting of 94°C for 1 min denaturation, 58°C for 1 min annealing, and 72°C for 1 min extension. The final extension profile is set at 72°C for 10 mins and the reaction is terminated and held at 4°C.

Table 1. *In silico*-designed primers featuring the following target genes: ORF1ab, S1, S2, E, and M

Target Gene	Primer Name	Primer Pair Sequence (5'→3')	Product size (bp)	GC content	Tm
ORF1ab	SARS-CoV-2_Var_01	*Forward primer 01* *ACCAATGTGCTATGAGGCCC*	818	55%	60.11
		Reverse primer 01 *CATCACCCAACTAGCAGGCA*		55%	60.04
S	SARS-CoV-2_Var_S1	*Forward primer S1* *CAAATCGCTCCAGGGCAAAC*	1362	55%	60.11
		Reverse primer S1 *GTGGCAAAACAGTAAGGCCG*		55%	60.04
S	SARS-CoV-2_Var_S2	*Forward primer S2* *GTCCTTCCCTCAGTCAGCAC*	655	60%	60.00
		Reverse primer S2 *GACTCCTTTGAGCACTGGCT*		55%	59.96
E,M	SARS-CoV-2_Var_EM1	*Forward primer EM1* *CGATTGTGTGCGTACTGCTG*		55%	59.01

Detection of the RT-PCR product

The amplicons were subjected to electrophoresis using a 1.0% agarose gel and were stained using gel red. The gels were quantified by using a nanodrop and were visualized under UV light using XR+ Gel Documentation System (Bio-rad, Japan).

Sequencing

Around 20 μL of the RT-PCR amplicons were packaged by wrapping parafilm wax around the lid of the PCR reaction tubes. The tubes were placed inside sealable bags and were sent for sequencing as per the service provider instructions (Macrogen Inc., South Korea).

Sequence analysis

The resulting sequencing results were visualized and analyzed using the BioEdit software. A consensus sequence was generated by aligning the forward and reverse sequence of the sample. Note that the reverse sequence is in the 3' to 5' orientation, thus the sequence should be flipped first to set the orientation to the 5' direction. After ensuring that both sequences were in the 5' direction, the sequence alignment was run using the ClustalW function of the software. After alignment, the sequence was edited by removing the lagging sequence strand first, followed by the removal of the leading sequence. Once completed, a consensus sequence was generated by using the Consensus Sequence generator function of the software.

BLAST search and lineage identification

A BLAST analysis was performed to determine the variant identity of the viral samples using the online platforms provided by the National Center for Biotechnology Information (NCBI) and the China National Center for Bioinformation. The consensus sequence of each sample was initially used as the query sequence for BLAST analysis, and the corresponding description, species identification, and accession number of the top hits were recorded. In cases where the consensus sequence failed to yield BLAST results, the forward sequencing read was used. If no significant match was obtained, the reverse sequence was reverse-complemented and subsequently analyzed. The resulting BLAST hits were documented accordingly. Using the recorded description and accession number, the SARS-CoV-2 variant was determined through the SARS-CoV-2 epidemiological lineage assignment tool available on the Cov-Lineages website.

RESULTS

A total of 16 samples were subjected to RNA extraction using PureLink™ Viral RNA/DNA kit and one-step RT-PCR assays. After extraction, the samples' concentration and purity were determined using NanoDrop™. The samples with good concentration and purity were included for further analysis. Pre-amplification RNA integrity and post-amplification product specificity were evaluated by agarose gel electrophoresis. Expected amplicon sizes for the DPSS primer sets were confirmed as follows: SARS-CoV-2_Var_S1 (1362 bp), SARS-CoV-2_Var_S2 (655 bp), SARS-CoV-2_Var_EM1 (658 bp), and SARS-CoV-2_Var_O1 (818 bp).

Figure 1 shows the post-PCR agarose gel electrophoresis result of samples 6, 7, 8, 10, 101, 128, 130, 133, 140, 143, 146, 147, 152, and 156 using SARS-CoV-2_Var_EM1 primer. Samples 7, 10, 143, 146, and 152 were the best samples in this primer showing a product size of more than 600 bp and a band greater than 60 ng. Only the PCR products meeting these criteria were selected for sequencing.

The raw sequence data were quality-checked, trimmed and assembled to generate consensus sequence and alighted to the SARS-CoV-2 reference genome and lineage classification performed using the Pango nomenclature system based on characteristic mutation profiles within the amplified regions. The assigned lineages are summarized in Table 3.

DISCUSSION

The results showed that the four (4) *in silico*-designed primers used for this study: S1, S2, E/M and Orf1ab had a sensitivity of 100% when used in combination to detect previously tested known nasopharyngeal/ oropharyngeal swab samples positive for SARS-CoV-2. Each primer had different sensitivities, with Orf1ab being the lowest at 58.82% and the E/M the highest at 90.91% (Table 4). The AGE for the E/M also had the highest number of bands noted (Figure 1). This is in contrast with other studies that

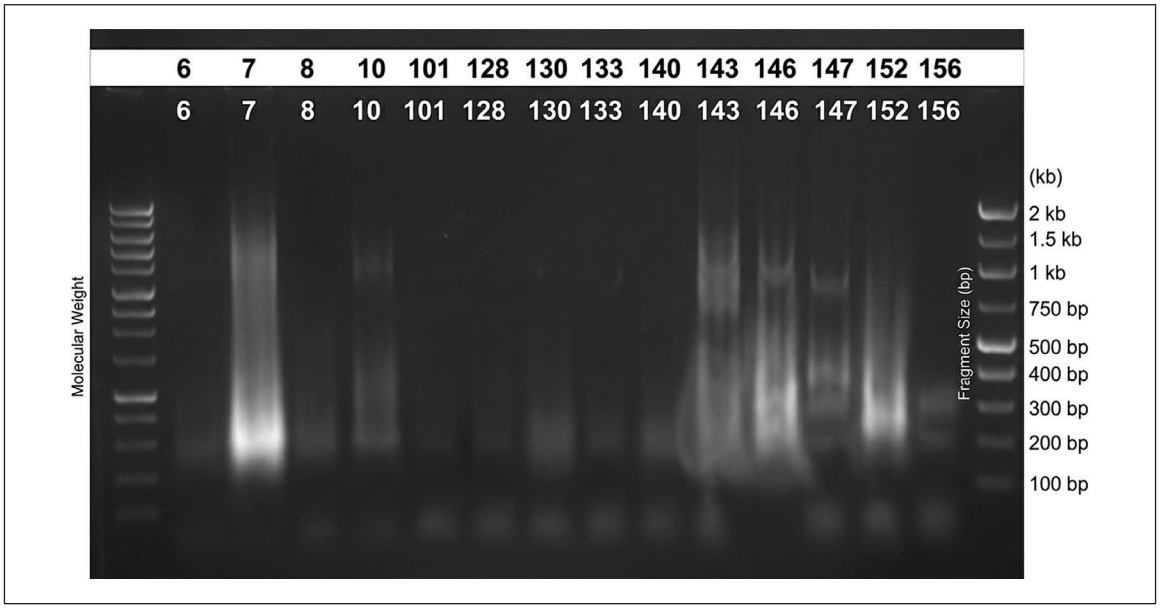

Figure 1. Post PCR agarose gel electrophoresis result for E/M primer (left to right: Samples 6, 7, 8, 10, 101, 128, 130, 133, 140, 143, 146, 147, 152 and 156).

show Orf1ab, N, and Rdrp as having the highest sensitivities among the different evaluated gene target primers.[12,15]

Designing primers for a virus that frequently mutates such as SARS-CoV-2 is challenging. Ideally, the target must be an area that is conserved in the genome so that it can still detect the virus. One possible explanation for the high sensitivity of E/M in our study can be attributed to the lack of mutation detected in the E/M gene from the samples included (Appendix 1). All the samples had multiple mutations in the S and ORF1ab proteins which is compatible with the variant classification of most samples under Omicron. This is similar to the study by Mentes et al., showed that the E gene had the lowest mutation rate from their experiment.[16] Further supporting this is a local study on the genome sequences in the Philippines during the start of the pandemic also showed that the most conserved among the different proteins is the envelope protein, 224 with 100% sequence similarity at both nucleotide and amino acid levels relative to the reference.[17]

The total number of mutations across the target regions, as well as the type (example: point mutation vs. deletion) can damage it significantly, leading to potential misclassification and affect the accuracy of diagnostics employing the primers. It can also affect primer and probe hybridization, causing amplification failure in real time polymerase chain reaction (RT-PCR), the gold standard for SARS-CoV-2 diagnosis. This is the reason why multiple primers or primer sets are used for SARS-CoV-2 diagnostics as recommended also by different studies[12,16,18] and supports our finding of high sensitivity for combined four primers rather than individual ones. Any conflicting results warrants repeat RT-PCR and agarose gel electrophoresis to exclude technical variability and persistent discordance maybe resolved by sequencing the amplified products. The sequencing data can be used to identify genomic regions and assess mutations that can affect primer binding sites that can explain amplification failures in certain targets.

The mutations in the samples were in the S and ORF1ab proteins, but the primers were still able to detect bands in the S protein but were not as successful in the ORF1ab (Appendix 1). No cross-reactivity was noted. The use of multiple targets therefore enhances overall diagnostic sensitivity by showing that there can be target-specific sequence variation rather than false positive or negative results through repeat testing and sequencing confirmation utilized by the DPPSS method.

There have been reports that variants of SARS-CoV-2 can affect the diagnostic accuracy of RT-PCR testing.[19] As mentioned, the mutations and other changes can affect the target regions of the primers designed to capture the virus. In this study, the sample sequencing revealed the different virus strains and classification (Table 2). The three WHO groups of COVID-19 virus variants are: variants of concern (VOCs), variants under monitoring (VUMs), and variants of interest (VOIs).[20] The most common VOC detected in this study was the Omicron, but others present during that time such as Delta were seen as well. This reflects the epidemiological data during the time the swabs were collected in 2021, with each primer showing specificity with different variants (Table 3). This also proves that this particular primer set can detect SARS-CoV-2 across different variants and mutations.

It could be recalled that three prominent waves have been noted since the COVID-19 pandemic was declared in 2020. The first wave was dominated by two VOC one after the other: alpha and delta until the first half of 2021.[20,21] Other variants with higher pathogenicity than alpha variants such as Beta (B.1.351) and Gamma (P.1) were also present during that time. The first Omicron (B.1.1.529) was reported in November 2021 from Botswana, South Africa. It was declared a VOC by the WHO because of its high transmissibility and virulence, related to the numerous mutations (26-32) in spike proteins, N terminal domain and receptor binding sites.[20] In the Philippines,

Table 2. Identified SARS-CoV-2 variants through BLAST using DPPSS primers

Sample	Primer Name		BLAST Result	SARS-CoV-2 Variant
AG	SARS-CoV-2_Var_S1	forward	**No significant similarity found.*	
		reverse		
		consensus	XBB.1.5.48	Omicron
AN	SARS-CoV-2_Var_S2	forward	FL.1.1 XBB.1.5.85	Omicron
		reverse	BA.2.3.1 BF.7.14.1	Omicron
		consensus	**No significant similarity found.*	
AZ	SARS-CoV-2_Var_S2	forward	FL.1 XBB.1.5.28	Omicron
		reverse	**No significant similarity found.*	
		consensus		
BO	SARS-CoV-2_Var_EM1	forward	**No significant similarity found.*	
		reverse		
		consensus	FL.1.5.1	Omicron
L	SARS-CoV-2_Var_EM1	forward	XBB.1.41.1 FL.1.5.1 EG.5.1.1	Omicron
		reverse	FY.3.1 XCF GP.2	Omicron
		consensus	**No significant similarity found.*	
Y	SARS-CoV-2-Var_S1	forward	BA.2.7 BA.2.1	Omicron
BG	SARS-CoV-2_Var_EM1	forward	XBB.1.28	Omicron
S	SARS-CoV-2_Var_O1	reverse	B.1.274	
AI	SARS-CoV-2_Var_S1	forward	BA.2.40.1	Omicron
		reverse	**No significant similarity found.*	
		consensus		
AM	SARS-CoV-2_Var_S2	forward	BA.5.2.2	Omicron
			AY.106	Delta
		reverse	BA.2.6.1 XBB.1.22.2	Omicron
		consensus	**No significant similarity found.*	
M	SARS-CoV-2_Var_EM1	forward	**No significant similarity found.*	
		reverse		
		consensus		
C	SARS-CoV-2_Var_S1	forward	**No significant similarity found.*	
		reverse		
		consensus		
G	SARS-CoV-2_Var_S2	forward	**No significant similarity found.*	
		reverse		
		consensus	FL.14 XBB.2.3.9 BA.5.1.38	Omicron
H	SARS-CoV-2_Var_S2	forward	**No significant similarity found.*	

the first cases of Omicron infection from the data of Philippine Genome Center (PGC) were reported in December of 2021 but unlike the worldwide data which showed predominance of BA.1 sublineage, there were more BA.2 cases observed locally.[22-24] Our findings showed concordance with this report.

Unlike the other VOCs with a convergent evolution pattern, Omicron is highly antigenically divergent. There is continuous antigenic drift that gives rise to sub lineages that have higher neutralization escape, decreasing the vaccination effectiveness.[25] This highlights the value of genomic surveillance even in the face of lower positivity rates, mortality and morbidity caused by the virus. Although VOC may still be responsive to current treatments and developed vaccines, there may be some standalone single nucleotide polymorphisms (SNPs) such as Spike mutation E484K that lowers sensitivity to antiviral drugs.[21]

While positivity rates were elevated during the height of the pandemic, they have since declined significantly in the current period. In a resource limited country, it is challenging to follow the international recommendations[26,27] on genomic SARS-CoV-2 monitoring which should be a threshold minimum of 2.5% or ideally 1% of a particular variant among all variants within one unit of time.[26] Sequencing was performed locally in several institutions (e.g., Philippine Genome Center), with a requirement of CT value of less than <30, positive for any SARS-CoV-2 specific target genes sent within 24 hours upon release of RT-PCR result.[28] There are higher CT values from positive samples detected in the current situation, further decreasing the

Table 3. Designed DPPSS primers specificity

		Primer	Name	Target Gene	Length	Tm	GC%	Specificity and date detected
1	fw	CAAATCGCTCCAGGGCAAAC	SARS-CoV-2_Var_S1	S	20	60.11	55	XBB.1.5 (Oct 2022) BA.2.1 (Late 2021) BA.2.7 (June 2022) BA.2.40.1 (Mid 2022)
	rv	GTGGCAAAACAGTAAGGCCG			20	60.04	55	
2	fw	GTCCTTCCCTCAGTCAGCAC	SARS-CoV-2_Var_S2	S	20	60.04	60	FL.1 (Late 2022) FL.1.1 (Mid 2023) XBB.1.5.28 (2022) XBB.1.5.85 (2022) BA.5.2.2 (Mid 2022) AY.106 (2020-Mid 2021)
	rv	GACTCCTTTGAGCACTGGCT			20	59.96	55	BA.2.3.1 (Early 2022) BA.2.6.1 (Early 2022) BF.7.14.1 (Late 2022) XBB.1.22.2 (Feb 2023)
3	fw	CGATTGTGTGCGTACTGCTG	SARS-CoV-2_Var_EM1	E & M	20	59.01	55	FL.1.5.1 (2023) EG.5.1.1 (2023) XBB.1.28 (Late 2022) XBB.1.41.1 (2023)
	rv	AGGTCCTTGATGTCACAGCG			20	60.04	55	FY.3.1 (2023) XCF (2022) GP.2 (2022)
4	fw	ACCAATGTGCTATGAGGCCC	SARS-CoV-2_Var_O1	ORF1ab	20	60.11	55	
	rv	CATCACCCAACTAGCAGGCA			20	60.04	55	B.1.274 (2020)

Note: The forward (fw) and reverse (rv) primer sequences are directed from 5′ to 3′.

Table 4. Designed DPPSS primers sensitivity

Primer	Sensitivity	Confidence Interval
S1	66.67%	47.19%-82.71%
S2	83.33%	62.62%-95.26%
E/M	90.91%	70.84%-98.88%
Orf1ab	58.82%	40.7%-75.35%
Combined 4 primers	100.00%	83.16%-100.00%

samples sent for genomic surveillance.[29] Hence, we might be missing out on important mutations and changes in the virus and the current epidemiologic data may not be representative of what is currently happening.

With locally designed primers such as the ones featured in our study, we highlight the importance of genomic surveillance on a broader geographical coverage to combat issues such as presence of immune escape variants,[29] as well as optimizing workflow for early detection of emerging variants. The DPPSS provides a rapid-cost effective and targeted surveillance tool for monitoring known variants in a resource limited setting. Compared with sequencing, this method is simple because it utilizes existing equipment available locally and in routine molecular laboratories such as PCR machines and electrophoresis, with a straightforward, simple workflow. Results can also be analyzed with a shorter turn-around time (TAT) because the scope of detection focuses on predefined genomic regions with no extensive bioinformatics requirement or need for high-throughput sequencing machine and extensive library preparations. In depth and comprehensive analysis can then be reserved for sequencing should the need arise.

Since Omicron, known to have S gene drop out, is currently the most widespread VOC, it is imperative that appropriately designed primers with other targets are included in the testing. Even though there are a lot of commercially available kits, some of them have undisclosed primer set sequences which may not be optimized leading to false positive results.[10] A study by Park et al., showed that under-optimized primer sets containing long and short dimer bands were present in commercial kits that could potentially cause false positive results. Continuous monitoring is needed to ensure that diagnostic tests can still detect SARS-CoV-2, maintaining the accuracy of kits available for detection.

The information from genomic data can also help in vaccine improvement and monitoring viral variability and genotypic features that impacts antigenicity, infectivity, pathogenicity and susceptibility to treatment and vaccines.[25] Aside from samples collected from patients, other surveillance methods such as wastewater monitoring[12] can also use the primer sets in this study to serve as an early warning for transmission trends. With recent Emerging/Re-emerging Infections Diseases (EREIDS) such as Nipah virus (NiV)[30] (reported in patients in Bangladesh, India who experienced severe neurological and respiratory symptoms) and epidemic-prone Water, Sanitation and Hygiene (WASH) pathogens, locally available methods for developing diagnostic tools must be available for swift response. This is applicable not just to emerging viral pathogens but also to other infectious agents of public health importance. We recommend that continuous surveillance must be performed to be prepared to manage any public health threat such as NiV and prevent re-emergence of SARS-CoV-2 pandemic.

CONCLUSION

The combined used of four *in silico*-designed primer sets (S1, S2, E/M and ORF1ab) demonstrated accurate detection of SARS-CoV-2 in previously confirmed RT-PCR-positive samples, including variants with S gene mutations such as Omicron. The multi-target design improves assay reliability despite the viral evolution.

The Direct PCR Product Sequencing Surveillance (DPPSS) approach provides a simple and cost-effective method that enables both detection and mutation monitoring. This makes it a practical tool for genomic surveillance in resource-limited settings. In the Philippines, DPPSS and similar Laboratory Developed Tests (LDTs) may be integrated into existing diagnostic workflows, subject to appropriate validation and regulatory compliance, to strengthen decentralized testing and support national surveillance efforts. It also ensures a stronger and prepared response to emerging global threats.

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STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

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