Detection of SARS-CoV-2 Delta, Omicron and XBB variant using colorimetric reverse-transcription loop-mediated isothermal amplification and specific primers

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Abstract. Even with global vaccination efforts, COVID-19 continues due to the emergent of new and more transmissible variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that evade immunity. Monitoring these variants is costly, often requiring whole-genome sequencing. Therefore, there is a need for more cost-effective and rapid alternatives for the identification of variant-specific samples. Reverse-transcription loop-mediated isothermal amplification (RT-LAMP) provides a more affordable and rapid method for detecting SARS-CoV-2 compared to other molecular tests. The present study designed colorimetric RT-LAMP assays that target mutations associated with specific SARS-CoV-2 lineages. These assays were tested on 347 confirmed SARS-CoV-2 RNA samples. The results revealed promising accuracy: 92.18% for Omicron-specific primers, followed by 80.64% for XBB and 71.57% for Delta. This assay effectively distinguishes the variants of concern, Delta, Omicron and XBB, without the use of costly equipment/facilities and does not require the specialized training of personnel. This development supports the use of RT-LAMP as a valuable tool in healthcare systems battling COVID-19.

Introduction

Since its emergence in 2019, COVID-19, driven by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread at a rapid pace. By March, 2024, the number of deaths reached 7 million worldwide, of which Malaysia recorded 37,349 (https://data.moh.gov.my/dashboard/covid-19). Of note, >1,560 SARS-CoV-2 lineages have been identified [PANGO Lineages network (https://cov-lineages.org/lineage_list.html; updated on December 4, 2024)]. Novel variants of concern (VOCs) with high mutation rates are a challenge. These variants, such as Delta and Omicron, become more infectious and evade vaccines to some degree, hindering pandemic control (1,2).

Of note, five major VOCs have emerged sequentially: Alpha (B.1.1.7/Q.*), Beta (B.1.351/), Gamma (P.1/P.1.*), Delta (B.1.617.2/AY.*) and Omicron (BA.1.1.529/BA.*). Delta, first detected in April, 2021, became dominant, but was then replaced by Omicron in December, 2021. The XBB variant was reported in Malaysia in September, 2022.

The monitoring of new variants through DNA sequencing is crucial for informing public health decisions. Canada leads in sequencing, analyzing almost 13% of positive cases. Despite having the 28th highest number of confirmed cases globally, Malaysia has sequenced <1% of these. (EpiCoV database in GISAID; https://www.gisaid.org/submission-tracker-global/). In developing nations, such as Myanmar, Libya and Azerbaijan, this issue is particularly acute, with sequencing coverage being <0.05%

While DNA sequencing is the gold standard, some researchers propose using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) with specific probes for more rapid and cost-effective variant surveillance (3). However, RT-qPCR may still be costly for some countries. Researchers are exploring affordable point-of-care tests for the diagnosis of COVID-19. RT-loop-mediated isothermal amplification (LAMP) shows promise as a reliable and rapid diagnostic tool (4,5). This method can detect the targeted region in a genome with considerable specificity and sensitivity in <40 min, without the necessity of a real-time thermocycler. The LAMP method utilizes a DNA polymerase and four primers to hybridize six regions on target DNA (6). An inner primer initiates LAMP by recognizing the sense and antisense strands of the target. Strand displacement DNA synthesis, driven by an outer primer,

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produces a single-stranded DNA template for further synthesis with the second inner and outer primers, forming a stem-loop structure. In the subsequent LAMP cycles, one inner primer binds to the loop of the product, initiating more synthesis and generating both the original and a new stem-loop DNA with an extended stem. Signal can be captured based on the presence of amplification through gel electrophoresis, colour change or fluorescence detection. An improved LAMP method utilizes two extra primers (termed forward loop and backward loop) which targets loop region (7). This six-primer LAMP reaction significantly reduces the signal generation to 15-30 min.

The present study developed RT-LAMP primers aimed at detecting specific mutations found in the Delta, Omicron and XBB variants. This novel assay successfully distinguished between samples containing these variants, providing an indirect method to monitor their prevalence. To the best of our knowledge, this is the first report on the use of RT-LAMP for VOC detection without additional probes or primers. The present study proves that RT-LAMP can be a more cost-effective alternative to DNA sequencing and RT-qPCR for variant surveillance.

Materials and methods

Isolation of RNA. All SARS-CoV-2 clinical samples examined in the present study were verified using whole-genome sequencing (WGS) (Fig. 1). RNA isolation from swabs was performed according to the manufacturer's guidelines (Nucleospin RNA Virus, Macherey-Nagel). The presence of SARS-CoV-2 virus was confirmed using RT-qPCR according to manufacturer's protocol (Viasure SARS-CoV-2 del 69/70, ORF1ab & N genes Detection kit, Cretest Biotec). Positive samples with Ct (threshold cycle) value ≤30 were selected and were used for WGS. Ct value is the cycle number where the amplification curve intersects with the threshold line and was determined automatically using real-time PCR software (CFX96 Touch Real-time PCR Thermal System, Bio-Rad Laboratories, Inc.). Sequencing libraries were created using the Illumina COVIDSeq assay kit and sequenced on Illumina MiSeq. Subsequently, the raw sequencing data underwent processing with bioinformatics tools to produce and identify assembled viral consensus sequences. Nextclade Web (https://clades.nextstrain.org/) is an online bioinformatics tool that performs alignment, mutation calling, clade assignment, phylogenetic placement and quality control checks. It was then used to evaluate the consensus sequences for quality assessment and to designate its nomenclature. The tool analyzes various sequence metrics, including non-N ambiguous bases, the number of nucleotide gaps (N's), and the positions of N runs in the genome. The consensus sequence (FASTA) files along with its associated metadata were uploaded to the public sequence database, Global Initiative on Sharing All Influenza Data (GISAID; https://www.gisaid.org).

Alignment of SARS-CoV-2 genome sequences and RT-LAMP primer design. Genome sequences of SARS-CoV-2 were downloaded from GISAID. The sequences includes representation from various continents and variants such as Alpha (B.1.1.7, Q.1-Q.8), Beta (B.1.351, B.1.351.2, B.1.351.3), Gamma (P.1, P.1.1, P.1.2), Delta (B.1.617.2), Omicron (B.1.1.529) and XBB (XBB.1.16.*). This comprehensive selection was used to pinpoint unique regions for RT-LAMP primer design, aiming for an assay that is as sensitive, specific, and accurate as possible for each targeted variant. The sequence quality assessment was made by implements a variety of quality control metrics such as missing data, mixed sites, private mutations, mutation clusters, frame shifts and stop codons with Nextclade (https://clades.nextstrain.org/). To perform a comparative analysis, the full genome sequences were aligned at the base level utilizing the MAFFT-L-INS-I multiple sequence alignment program (https://mafft.cbrc.jp/alignment/software/). The Wuhan-Hu-1 sequence was set as the reference sequence to be aligned with. The selection of primer binding sites was based on the unique regions specific for each SARS-CoV-2 variant. The PrimerExplorer V5 program (available at https://primerexplorer.jp/e/) was then used to design each RT-LAMP primer sets (each containing four to six primers). The sequences of all primer sets are listed in Table I. The hybridization region for each LAMP primers on target variants is illustrated in Fig. 2.

The oligonucleotides were purchased through IDT. All the designed primer sets were tested on synthetic SARS-CoV-2 RNA (Twist Bioscience synthetic RNA control), non-template control (NTC) or verified patient RNA sample. The primer sets with optimal performance were selected for further testing and analysis. In a reaction volume (25 μl), the primer concentration (10X) were settled as follows: 2 μM for F3 and B3, 4 μM for LF and LB, and 16 μM for FIP and BIP.

Control RNA. Synthetic SARS-CoV-2 RNA for SARS-CoV-2 variants was purchased from Twist Bioscience [Control 14 B.1.1.7_710528 (Alpha B.1.1.7), Control 16 EPI_ISL_678597 (Beta B.1.351), Control 17 EPI_ISL_792683 (Gamma P.1), Control 23 EPI_ISL_1544014 (Delta B.617.2)]. RNA samples were diluted in nuclease-free water to 1,000 copies/μl, aliquoted and kept in -80˚C as working stock.

Colorimetric RT-LAMP assay. A WarmStart Colorimetric RT-LAMP 2X Master Mix (New England Biolabs) was used to perform RT-LAMP-based detection of SARS-CoV-2. The reaction mixture is composed of Master Mix (12.5 μl), 10X primer mix (2.5 μl), RNA sample (2.5 μl) and dH_2O up to 25 μl. The mixture was incubated in a heating block at 60˚C for 60 min or until a color changes appeared. The amplified product was evaluated visually by color, where a yellow hue indicates the presence of the target variant and a pink hue shows its absence.

Collection of clinical samples. Multiple validations were conducted on real clinical specimens, which were left-over RNA-extracted samples from an in-house project on SARS-CoV-2 genomic surveillance. A total of 347 RNA samples, validated as either positive or negative for Delta, Omicron, and XBB SARS-CoV-2, were tested simultaneously with the colorimetric RT-LAMP assay developed in this study. The specificity was determined by testing with another SARS-CoV-2 variant.

The National Institutes of Biotechnology Malaysia confirmed that no ethics approval or patient consent were required for the present study due to the following reasons: The study was conducted as part of a government-funded initiative during the COVID-19 pandemic, which prioritized rapid response to public health needs. The samples used for

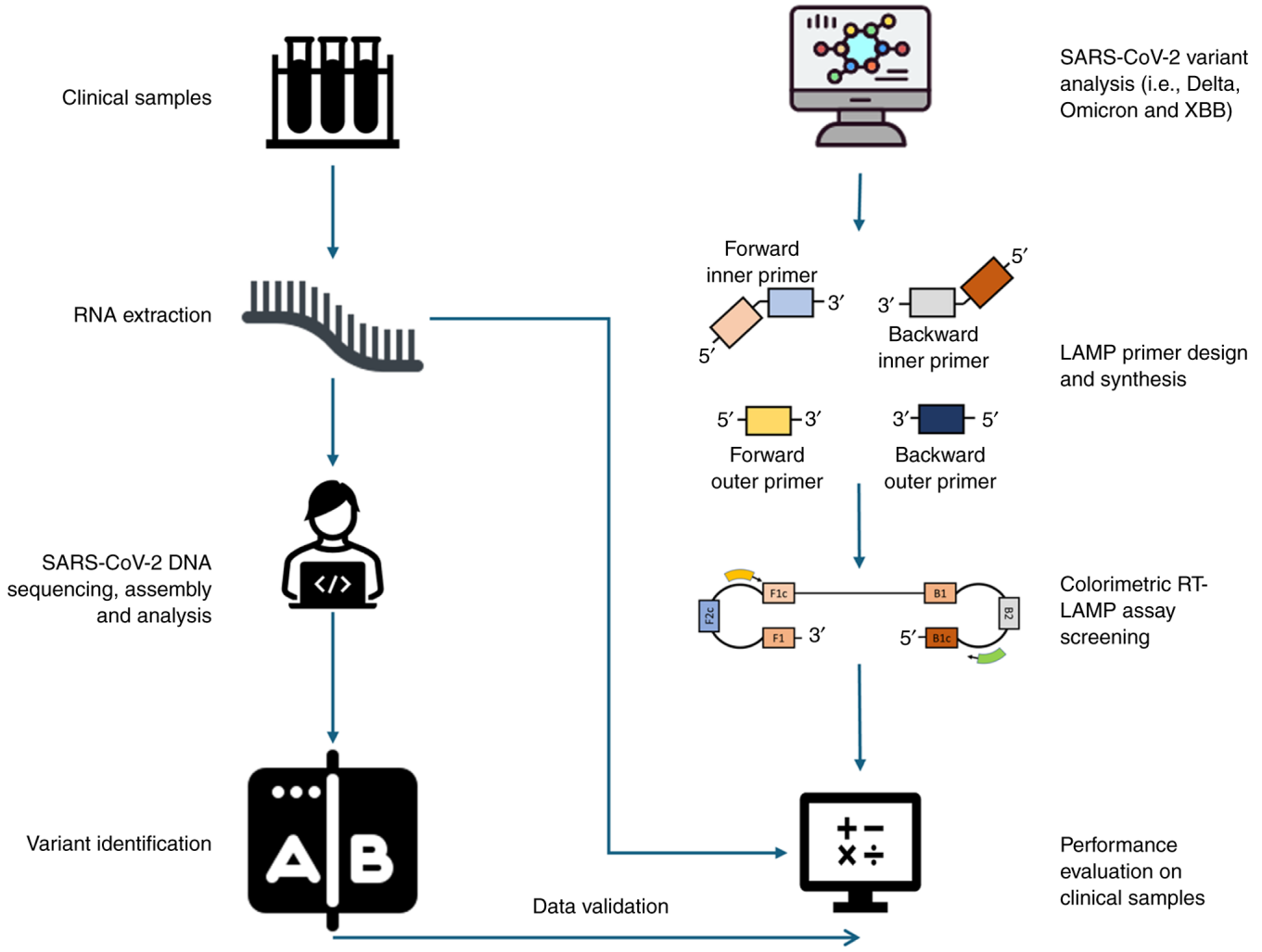

Figure 1. Schematic overview of RT-LAMP assay development for SARS-CoV-2 variants. RT-LAMP, reverse-transcription loop-mediated isothermal amplification; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

the project were provided by government hospitals specifically for surveillance and sequencing purposes, in alignment with national public health objectives. Under such circumstances, the project fell within the scope of public health surveillance, which is often exempt from individual consent requirements. Additionally, the study adhered to strict data protection protocols, ensuring that all samples were anonymized and used solely for the purpose of developing diagnostic methods to combat the pandemic.

Statistical analysis. Diagnostic studies were analyzed based on the extracted true positive (TP), false positive (FP), false negative (FN) and true negative (TN) data. The diagnostic performance of RT-LAMP was evaluated by examining the accuracy, sensitivity and specificity. The equations for calculations were as follows: Accuracy=(TP + TN)/n; sensitivity=TP/(TP + FN); specificity=TN/(TN + FP), where n=the total no. of samples.

Results

RT-LAMP primer performance. Cross-reactivity against all available synthetic RNA VOCs was performed to assess the performance of the specific LAMP primers in targeting the respective variants. As illustrated in Fig. 3A, delta- and omicron-specific primers exhibited a positive reaction only in the presence of targeted VOC. At the time of the experiment, no VOC XBB synthetic RNA was available. Nonetheless, XBB-specific primers did not exhibit a positive reaction against all other tested synthetic RNA samples. Subsequent test using verified XBB sample showed positive reaction (data not shown).

To assess the optimal reaction time, RT-LAMP was performed at different time points from 10 to 60 min. The optimal reaction time was 30 min for all tested VOC-specific primers (Fig. 3B). A reaction time >60 min was not tested due to the limitation of LAMP reactions where non-specific reactions would be observed, as reported by previous research (8).

RT-LAMP assays on clinical samples. The performance of the Delta-, Omicron- and XBB-specific primer sets were tested on clinical specimens and the results are presented in Table II. A total of 347 clinical specimens were tested (203 positive and 144 negative samples). These samples were validated as positive for SARS-CoV-2 through RT-qPCR and sequencing. The reactions were performed in duplicate due to the limited

Table I. Primer sequences used in the present study.

Variant	Primer set	Target mutation	Sequence (5'-3')
Delta	LRTK	S:L452R and S:T478K	F3: CAAACTGGAAAGATTGCTGAT
			B3: ACCATATGATTGTAAAGGAAAGT
			FIP: **C**GGTAATTATAATTACCACCAACCTTGATTTTACAGGCTGCGT
			BIP: GTTTAGGAAGTCTAATCTCAAACCTAGGCCCCATTACAAGGTT
			LB: TTCAACTGAAATCTATC
Omicron	Set5+	S:E452A	F3: CTGTATAGATTGTTTAGGAAGTCT
			B3: TGGTGCATGTAGAAGTTCAA
			FIP: **G**CAACACCATTACAAGGTTTGTTACTTTTGAGAGAGATATTTC AACTGA
			BIP: TTACGATCATATAGTTTCCGACCCAAGAAAGTACTACTACTCT GTATGG
			LF: CCGGCCTGATAGATT
			LB: CTTATGGTGTTGGTCA
XBB	XBB	A19326G	F3: GACAAATTCACAGATGGTGTA
			B3: CTGTCAGAGTAATAGAAAAATGGTA
			FIP: AGGTTAGATAGCACTCTAGTGTCAAGCCTATTTTGGAATTGCA ATGT
			BIP: TAACTTGCCTGGTTGTGATGGTAAAAGCATTTTATCAAAAGCC
			LF: GGAATTAGCAGGATATCTATC
			LB: GCAGTTTGTATGTAAATAAAC

All the primers were designed using the PrimerExplorer V5 program. The bold letter represents the target mutation on respective variant. For primer set LRTK, only five primers were suggested by PrimerExplorer V5. All primer sets were tested in separate reactions on different SARS-CoV-2 variants. The single underline indicates the 5' region of inner primer FIP and BIP; the double underline: 3' indicates the region of inner primers FIP and BIP. F3, forward outer; FIP, forward inner; LF, loop forward; B3, backward outer; BIP, backward inner; LB, loop backward.

amount of sample volume. As shown in Table II, the Ct values (quantified using RT-qPCR), the variant identity (based on sequencing result and categorization according to the Pangolin lineage assigner (https://cov-lineages.org/resources/pangolin.html), the result for the two RT-LAMP replicates, and the test outcome. True positive (or true negative) were classified as having both replicates to be correct, and false positive (or false negative) as either one replicate to be incorrect.

The clinical performance of the Delta-, Omicron- and XBB-specific primer sets is summarized in Table III. Omicron-specific LAMP primers performed exhibited the optimal performance, with a sensitivity of 89.47%, specificity of 100% and an accuracy of 92.18%. The Delta-specific LAMP primers had the highest sensitivity of 100%, but with a very low specificity of 43.75% and a final accuracy of 71.57%. The XBB-specific LAMP primers scored a sensitivity of 91.80%, a specificity of 69.84% and an accuracy of 80.64%.

Discussion

Throughout the pandemic, SARS-CoV-2 has been evolving through mutations in its genetic code. These mutations can endow the virus certain advantages, such as spreading more easily or evading our immune defenses (2). Variants with these beneficial mutations, known as VOCs tend to become more prevalent in the population. To remain ahead of the virus, it is crucial to track new variants with the potential to spread rapidly. WGS is a powerful tool for identifying these new variants. However, researchers have also discovered specific mutations that are unique to each VOC. These unique mutations function as a fingerprint, allowing scientists to identify the variant without needing full genetic sequencing.

The geographical distribution of SARS-CoV-2 variants has been uneven, with some regions experiencing rapid and successive waves of different variants, while others have had less data due to limited sequencing. The Delta variant was particularly widespread, predominating in Africa, Asia, Europe, North America and Oceania by July, 2021 (9). In South America, the Gamma variant remained dominant for a time, before being overtaken by the Delta variant. The emergence of the Omicron variant then rapidly shifted the global landscape again. However, the detection and tracking of these variants has been heavily influenced by sequencing capacity, with countries such as the United Kingdom having higher sequencing coverage compared to countries such as Brazil, where the sequencing of positive cases is markedly lower (8). This discrepancy in genomic surveillance highlights the challenge of monitoring variants in real-time.

The lack of sequencing capacity in a number of low- and middle-income countries creates a significant gap in the understanding of variant spread and evolution (10). This limitation means that data from these regions may be underrepresented

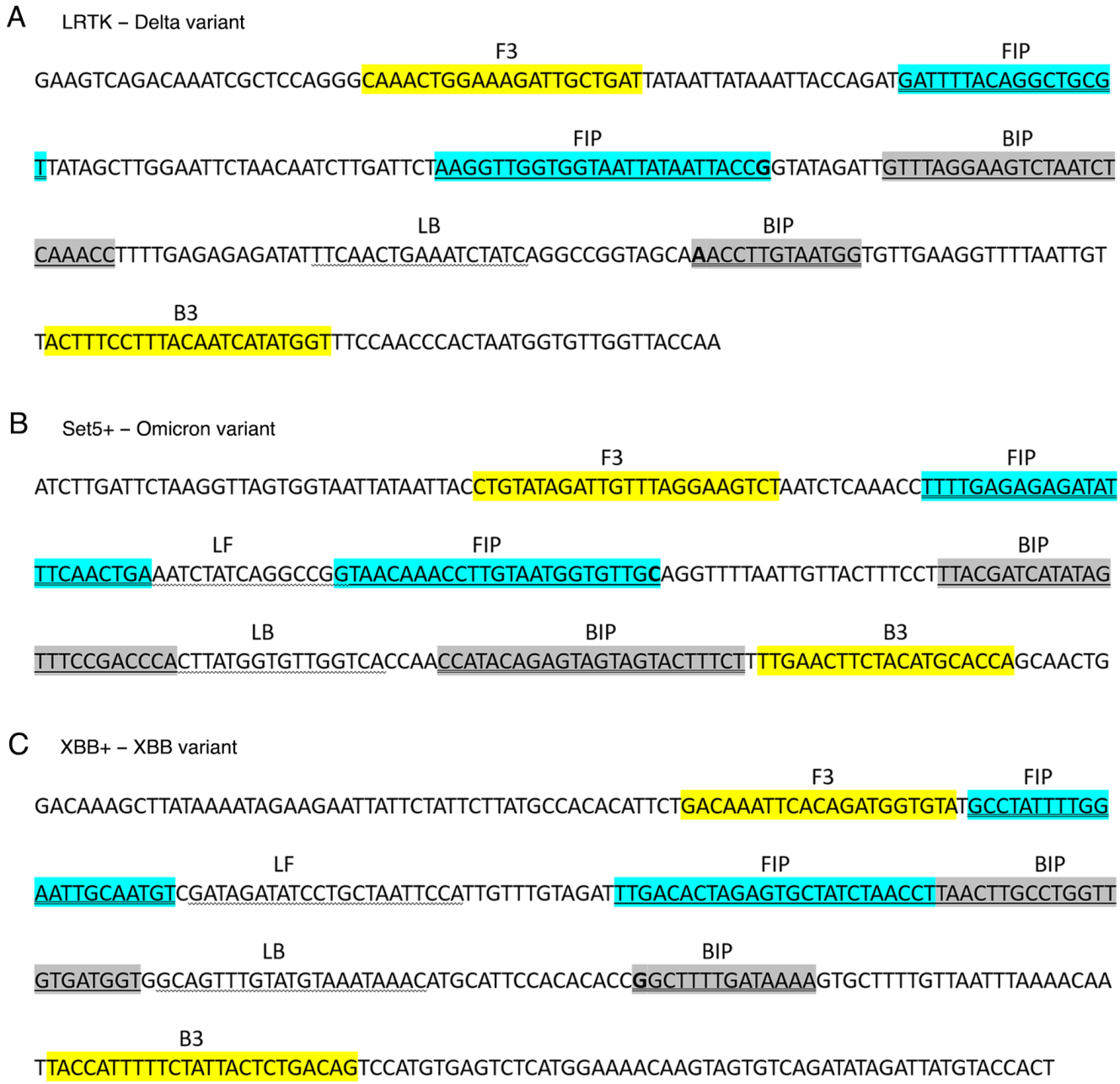

Figure 2. LAMP primer target location for SARS-CoV-2 variants. (A) Delta, (B) Omicron and (C) XBB. Yellow color indicates outer primers F3 and B3; blue indicates inner primer FIP; grey indicates inner primer BIP. The single underline indicates the 5' region of the inner primers FIP and BIP; the double underline indicates the 3' region of the inner primers FIP and BIP; the wavy underline indicates the loop primers LF and LB. Please refer to Table I for the LAMP primer sequences. LAMP, loop-mediated isothermal amplification; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

in global databases, such as GISAID, potentially leading to an incomplete picture of global variant dynamics (9). The uneven distribution of sequencing capacity also means that some countries may experience surges in cases due to a variant, but have limited ability to understand which variant is responsible. There is a need for more accessible and affordable methods for variant detection, such as RT-LAMP, to be deployed in these regions to complement limited sequencing capacity and to help ensure effective public health responses and control measures.

While some recent research has focused on using RT-LAMP to detect all SARS-CoV-2 variants regardless of strain (11,12), this approach offers limited value for tracking specific variants in epidemiological studies. However, RT-LAMP has exhibited promise in identifying specific variants. For example, Sherril-Mix *et al* (13) developed a method using molecular beacons to detect the Alpha variant (B.1.1.7) by targeting the S1Δ69-70 deletion in its genome. Another study by Yang *et al* (14) presented an RT-LAMP genotyping method that specifically identifies Delta variants by analyzing the R203M mutation in the N gene. This method achieved high accuracy without requiring additional probes, but relied on calculating the Ct ratio between results from two separate reactions per sample. Building on this progress, dos Santos *et al* (8) proposed a concept for an RT-LAMP assay targeting mutations C21614T (S:L18F) and C21638T (S:P26S) specific to Gamma variants and their descendants. This

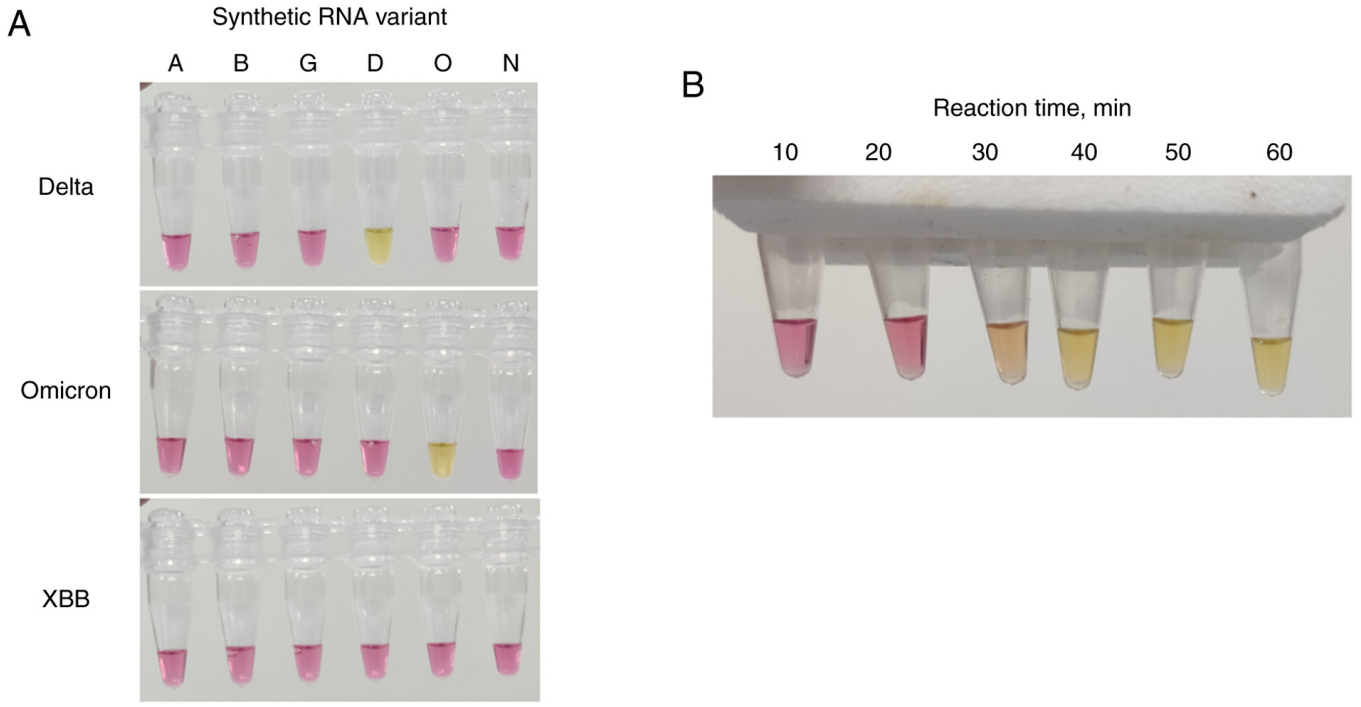

Figure 3. Specificity of the RT-LAMP reaction incubated at 65˚C. A separate RT-LAMP reaction for performed for each primer set. (A) RT-LAMP amplification using specific primer sets on different RNA samples from different lineages of SARS-CoV-2 as the target. The WarmStart Colorimetric kit uses a pH indicator that turns from pink to yellow in the presence of amplicons. (B) Determination of minimum reaction time for RT-LAMP reaction. Results are shown only for the Omicron-specific LAMP primer and synthetic RNA sample. The lanes are labeled as follows: A, VOC Alpha; B, VOC Beta; G, VOC Gamma; D, VOC Delta; O, VOC Omicron; N, non-template control using H_2O. RT-LAMP, reverse-transcription loop-mediated isothermal amplification; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VOC, variant of concern.

method avoids complex equipment and additional probes often required in conventional quantitative LAMP protocols. The present study aimed to take this a step further by introducing specific primer sets designed to detect the Delta, Omicron and XBB variants based on their unique mutations (S: L452R, S: T478K, S: E452A and A19326G).

In the present study, a total of 23 sets of LAMP primes were designed and screened for their performance against targeted SARS-CoV-2 variants (i.e. Delta, Omicron and XBB). Only three sets of LAMP primers exhibited promising performance against its target (Table I). This suggests a limited capability of openly available software tools (i.e., PrimerExplorerV5) and a poor understanding in LAMP primer design. One should be prepared to design and screen a handful of LAMP primers to increase the chances of obtaining a few useable candidates. A notable contribution of the present study is that it describes novel RT-LAMP primers that can distinguish between COVID-19 samples carrying mutations specific to the VOCs, Delta, Omicron and XBB (and its descendants). These mutations function as markers for identifying these VOCs with an accuracy of 71 to 92% in samples with a Ct value ≤25). Notably, the assay even performs well for samples with a Ct value as high as 30. This accuracy is comparable to that reported by dos Santos *et al* (8) (91.11%), highlighting the potential of these primers for SARS-CoV-2 lineage detection.

The recent study by Alhamid *et al* (15) demonstrated an optimal LAMP performance on SARS-CoV-2 detection by removing outer primer (i.e., LF) in the LAMP reaction. This five-primer LAMP assay avoids misamplification for up to 120 min, thus significantly preventing false positive results. Coincidently, primer set LRTK (Table I) in the present study is also a five-primer LAMP system. Outer primer LF was not suggested by PrimerExplorerV5 for primer set LRTK due to the low scoring of LF candidates. Nevertheless, primer set LRTK was tested and exhibited a good performance against the Delta variant detection. However, LAMP reaction using primer set LRTK was not performed and its misamplification ability was not determined. While the study by Alhamid *et al* (15) and the present study demonstrated the practicality of five-primer LAMP reaction, further studies need to be performed on different target locations (for the same gene) and different genes to prove its usefulness.

The RT-LAMP assay is proving useful for monitoring emerging disease variants. It offers sensitivity comparable to traditional sequencing, the current gold standard. In the present study, RT-LAMP successfully distinguished the majority of samples with a Ct value ≤25, with some Ct values reaching 30. Sequencing functions best with samples that have lower Ct values as they provide more genetic material, leading to more accurate variant identification. This suggests that the RT-LAMP assay from the present study could be a valuable screening tool in areas with limited sequencing capabilities.

A key advantage of RT-LAMP for variant identification is its speed and affordability compared to other techniques. It has been demonstrated that RT-LAMP costs significantly less per sample, at least 50-fold more economical than next-generation sequencing (NGS) and 4.6-fold more economical than RT-qPCR (8). Furthermore, RT-LAMP boasts a more rapid

Table II. Comparison between sequencing and RT-LAMP results for clinical samples.

			Sequencing results[d]		RT-LAMP results[e]		
Sample[a]	GISAID ID[b]	Ct[c]	Variant	Pango lineage	Replicate 1	Replicate 2	Outcome[f]
Delta							
GS0677	EPI_ISL_11899931	16.11	Delta	AY.59	+	+	TP
GS0679	EPI_ISL_11899933	15.74	Delta	AY.59	+	+	TP
GS0681	EPI_ISL_11899934	20.39	Delta	AY.79	+	+	TP
GS0682	EPI_ISL_11899935	24.49	Delta	AY.79	+	+	TP
GS0683	EPI_ISL_11899936	25.45	Delta	AY.79	+	+	TP
GS0685	EPI_ISL_11899938	20.08	Delta	AY.75	+	+	TP
GS0686	EPI_ISL_11899939	20.25	Delta	AY.79	+	+	TP
GS0688	EPI_ISL_11899940	22.91	Delta	AY.59	+	+	TP
GS0689	EPI_ISL_11899941	24.39	Delta	AY.59	+	+	TP
GS0690	EPI_ISL_11899942	27.62	Delta	AY.59	+	+	TP
GS0691	EPI_ISL_11899943	24.93	Delta	AY.59	+	+	TP
GS0694	EPI_ISL_11899945	21.38	Delta	AY.23	+	+	TP
GS0695	EPI_ISL_11899946	23.02	Delta	AY.79	+	+	TP
GS0696	EPI_ISL_11899947	21.25	Delta	AY.59	+	+	TP
GS0697	EPI_ISL_11899948	17.66	Delta	AY.23	+	+	TP
GS0698	EPI_ISL_11899949	20.02	Delta	AY.59	+	+	TP
GS0730	EPI_ISL_11899957	20.96	Delta	AY.79	+	+	TP
GS0762	EPI_ISL_11899967	22.43	Delta	AY.79	+	+	TP
GS0765	EPI_ISL_11899970	24.8	Delta	AY.76	+	+	TP
GS0773	EPI_ISL_11899973	24.67	Delta	AY.59	+	+	TP
GS0774	EPI_ISL_11899974	16.69	Delta	AY.59	+	+	TP
GS0775	EPI_ISL_11899975	16.29	Delta	AY.59	+	+	TP
GS0786	EPI_ISL_11899982	15.50	Delta	AY.59	+	+	TP
GS0791	EPI_ISL_11899985	17.04	Delta	AY.79	+	+	TP
GS0792	EPI_ISL_11899986	19.02	Delta	AY.59	+	+	TP
GS0794	EPI_ISL_11899988	22.51	Delta	AY.79	+	+	TP
GS0796	EPI_ISL_11899990	21.33	Delta	AY.79	+	+	TP
GS0797	EPI_ISL_11899991	19.38	Delta	AY.79	+	+	TP
GS0798	EPI_ISL_11899992	17.18	Delta	AY.79	+	+	TP
GS0799	EPI_ISL_11899993	16.61	Delta	AY.79	+	+	TP
GS0800	EPI_ISL_11899994	19.11	Delta	AY.79	+	+	TP
GS0801	EPI_ISL_11899995	14.89	Delta	AY.59	+	+	TP
GS0802	EPI_ISL_11899996	20.88	Delta	AY.59	+	+	TP
GS0804	EPI_ISL_11899998	19.35	Delta	AY.79	+	+	TP
GS0806	EPI_ISL_11899999	28.7	Delta	AY.59	+	+	TP
GS0809	EPI_ISL_11900000	26.89	Delta	AY.79	+	+	TP
GS0821	EPI_ISL_11900009	31.07	Delta	AY.79	+	+	TP
GS0829	EPI_ISL_13203067	21.18	Delta	AY.79	+	+	TP
GS0846	EPI_ISL_11900013	22.77	Delta	AY.79	+	+	TP
GS0900	EPI_ISL_11900033	24.55	Delta	AY.68	+	+	TP
GS0903	EPI_ISL_11900034	20.99	Delta	AY.59	+	+	TP
GS0904	EPI_ISL_11900035	26.29	Delta	AY.68	+	+	TP
GS0905	EPI_ISL_11900036	26.98	Delta	AY.79	+	+	TP
GS0906	EPI_ISL_11900037	15.06	Delta	AY.59	+	+	TP
GS0907	EPI_ISL_11900038	27.11	Delta	AY.68	+	+	TP
GS0908	EPI_ISL_11900039	25.4	Delta	AY.59	+	+	TP
GS0910	EPI_ISL_11900040	18.69	Delta	AY.59	+	+	TP
GS1122	EPI_ISL_13202995	25.50	Omicron	BA.1.1	+	+	FP
GS1121	EPI_ISL_13202994	17.92	Omicron	BA.1.1	+	-	FP

Table II. Continued.

Sample[a]	GISAID ID[b]	Ct[c]	Sequencing results[d]		RT-LAMP results[e]		Outcome[f]
			Variant	Pango lineage	Replicate 1	Replicate 2	
GS1119	EPI_ISL_13202993	25.17	Omicron	BA.1.1	-	+	FP
GS1118	EPI_ISL_13202992	25.88	Omicron	BA.1.1	+	-	FP
GS1128	EPI_ISL_13202999	18.16	Omicron	BA.1.15	-	-	TN
GS1117	EPI_ISL_13202991	26.53	Omicron	BA.1	-	-	TN
GS1116	EPI_ISL_13202990	19.46	Omicron	BA.1.1	-	-	TN
GS1113	EPI_ISL_13202987	18.70	Omicron	BA.1.1	+	-	FP
GS1112	EPI_ISL_13202986	20.50	Omicron	BA.2.10	-	-	TN
GS1111	EPI_ISL_13202985	25.14	Omicron	BA.1.14	+	+	FP
GS1110	EPI_ISL_13203088	25.47	Omicron	BA.1.1	+	+	FP
GS1109	EPI_ISL_13203087	25.95	Omicron	BA.1.1	-	+	FP
GS1108	EPI_ISL_13203086	20.20	Omicron	BA.1.1	-	-	TN
GS1107	EPI_ISL_13203085	24.21	Omicron	BA.1.1	+	+	FP
GS1106	EPI_ISL_13203084	20.40	Omicron	BA.1.1	-	-	TN
GS1105	EPI_ISL_13203083	15.91	Omicron	BA.1.1	+	+	FP
GS1104	EPI_ISL_13203082	16.13	Omicron	BA.1.1	+	-	FP
GS1103	EPI_ISL_13203081	19.20	Omicron	BA.1.1	-	+	FP
GS1102	EPI_ISL_13203080	25.02	Omicron	BA.1.1	-	-	TN
GS1101	EPI_ISL_13203079	18.37	Omicron	BA.2	-	-	TN
GS1100	EPI_ISL_13203078	24.09	Omicron	BA.1.1	+	+	FP
GS1099	EPI_ISL_13203077	24.16	Omicron	BA.1.1	+	-	FP
GS1098	EPI_ISL_13203076	23.65	Omicron	BA.2	+	+	FP
GS0924	EPI_ISL_11900050	29.69	Omicron	BA.1.1	+	+	FP
GS0921	EPI_ISL_11900048	30.12	Omicron	BA.1.1	+	+	FP
GS0920	EPI_ISL_11900047	21.72	Omicron	BA.1.1	+	-	FP
GS0919	EPI_ISL_11900046	20.45	Omicron	BA.1.1	-	-	TN
GS0918	EPI_ISL_11900045	17.68	Omicron	BA.2	-	-	TN
GS0917	EPI_ISL_11900044	22.27	Omicron	BA.2	+	+	FP
GS0898	EPI_ISL_11900032	22.72	Omicron	BA.1	-	-	TN
GS0892	EPI_ISL_11900031	18.46	Omicron	BA.1.1	+	-	FP
GS0891	EPI_ISL_11900030	26.48	Omicron	BA.1.1	+	-	FP
GS0887	EPI_ISL_11900029	27.01	Omicron	BA.1.1	+	+	FP
GS0884	EPI_ISL_11900028	21.13	Omicron	BA.1.1	+	-	FP
GS0883	EPI_ISL_11900027	23.1	Omicron	BA.1.1	+	+	FP
GS0882	EPI_ISL_11900026	25.84	Omicron	BA.1.1	-	-	TN
GS0881	EPI_ISL_11900025	25.71	Omicron	BA.1.1	+	+	FP
GS0877	EPI_ISL_11900024	20.66	Omicron	BA.2	-	+	FP
GS0871	EPI_ISL_11900023	29.00	Omicron	BA.1.1	-	-	TN
GS0868	EPI_ISL_11900022	19.29	Omicron	BA.1	+	+	FP
GS0865	EPI_ISL_11900021	17.38	Omicron	BA.2	-	-	TN
GS0864	EPI_ISL_11900020	15.86	Omicron	BA.2	-	-	TN
GS0863	EPI_ISL_11900019	19.58	Omicron	BA.2	-	-	TN
GS0862	EPI_ISL_11900018	21.14	Omicron	BA.1.1	-	-	TN
GS0861	EPI_ISL_11900017	18.89	Omicron	BA.2	-	-	TN
GS0853	EPI_ISL_11900016	17.88	Omicron	BA.1.1	-	-	TN
GS0851	EPI_ISL_11900015	18.28	Omicron	BA.2	-	-	TN
GS0849	EPI_ISL_11900014	24.76	Omicron	BA.1.1	-	-	TN
Omicron							
GS0940	EPI_ISL_11976171	17.02	Omicron	BA.2	+	+	TP
GS0931	EPI_ISL_11900056	29.58	Omicron	BA.1.1	+	+	TP
GS0930	EPI_ISL_11900055	25.31	Omicron	BA.2	+	+	TP

Table II. Continued.

Sample[a]	GISAID ID[b]	Ct[c]	Sequencing results[d]		RT-LAMP results[e]		Outcome[f]
			Variant	Pango lineage	Replicate 1	Replicate 2	
GS0929	EPI_ISL_11900054	25.27	Delta	AY.79	-	-	TN
GS0928	EPI_ISL_11900053	23.26	Omicron	BA.1.1	+	+	TP
GS0927	EPI_ISL_11900052	14.59	Omicron	BA.1.1	+	+	TP
GS0925	EPI_ISL_11900051	16.62	Omicron	BA.1	+	+	TP
GU0139	EPI_ISL_13651380	25.03	Omicron	BA.1.1	+	-	FN
GU0138	EPI_ISL_13651379	22.43	Omicron	BA.2.3	+	+	TP
GU0137	EPI_ISL_13651378	17.24	Omicron	BA.2	+	+	TP
GU0136	EPI_ISL_13651377	17.13	Omicron	BA.1.1	+	+	TP
GU0135	EPI_ISL_13651376	26.75	Omicron	BA.2	+	+	TP
GU0134	EPI_ISL_13651375	26.51	Omicron	BA.2.10	+	+	TP
GU0133	EPI_ISL_13651374	19.09	Omicron	BA.1.1	+	+	TP
GU0132	EPI_ISL_13651373	24.9	Omicron	BA.1.1	+	+	TP
GU0131	EPI_ISL_13651372	20.32	Omicron	BA.2.3	+	+	TP
GU0130	EPI_ISL_13651371	23.61	Omicron	BA.2.3	+	+	TP
GU0129	EPI_ISL_13651370	24.37	Omicron	BA.2.3	+	+	TP
GU0128	EPI_ISL_13651369	25.13	Omicron	BA.2.3	+	+	TP
GU0127	EPI_ISL_13651368	17.14	Omicron	BA.2	+	+	TP
GU0126	EPI_ISL_13651367	18.96	Omicron	BA.2.10	+	+	TP
GU0125	EPI_ISL_13651366	20.55	Omicron	BA.2	+	+	TP
GU0124	EPI_ISL_13651365	18.36	Omicron	BA.2.3	+	+	TP
GU0123	EPI_ISL_13651364	26.36	Omicron	BA.2.10	+	+	TP
GU0122	EPI_ISL_13651363	28.94	Omicron	BA.2	+	+	TP
GU0121	EPI_ISL_13651362	27.19	Omicron	BA.1.1	+	+	TP
GU0120	EPI_ISL_13651361	18.29	Omicron	BA.1.1	+	+	TP
GU0119	EPI_ISL_13651360	21.87	Omicron	BA.2	+	+	TP
GU0118	EPI_ISL_13651359	25.15	Omicron	BA.2	+	+	TP
GU0117	EPI_ISL_13651358	21.7	Omicron	BA.1.1	+	+	TP
GU0116	EPI_ISL_13651357	20.44	Omicron	BA.2	+	+	TP
GU0115	EPI_ISL_13651356	23.13	Omicron	BA.2.3	-	+	FN
GU0114	EPI_ISL_13651355	18.22	Omicron	BA.2.10	+	+	TP
GU0216	EPI_ISL_13651400	14.69	Omicron	BA.5	+	+	TP
GU0215	EPI_ISL_13651399	18.62	Omicron	BA.2	+	+	TP
GU0214	EPI_ISL_13651398	19.48	Omicron	BA.2	+	+	TP
GU0213	EPI_ISL_13651397	22.85	Omicron	BA.2	+	+	TP
GU0212	EPI_ISL_13651396	25.02	Omicron	BA.2.3	+	+	TP
GU0211	EPI_ISL_13651395	17.89	Omicron	BA.5	+	+	TP
GU0210	EPI_ISL_13651394	16.19	Omicron	BA.2	+	+	TP
GU0209	EPI_ISL_13651393	17.39	Omicron	BA.2.3	+	+	TP
GU0208	EPI_ISL_13651392	15.04	Omicron	BA.2.3	+	+	TP
GU0207	EPI_ISL_13651391	21.9	Omicron	BA.5	-	-	FN
GU0206	EPI_ISL_13651390	14.64	Omicron	BA.2.40.1	+	+	TP
GU0205	EPI_ISL_13651389	21.57	Omicron	BA.2	+	+	TP
GU0204	EPI_ISL_13329647	26.02	Omicron	BA.2.3	+	+	TP
GU0203	EPI_ISL_13329646	20.91	Omicron	BA.2	+	+	TP
GU0197	EPI_ISL_13203015	21.27	Omicron	BA.2	+	+	TP
GS0849	EPI_ISL_11900014	24.76	Omicron	BA.1.1	+	+	TP
GS0851	EPI_ISL_11900015	18.28	Omicron	BA.2	+	+	TP
GS0853	EPI_ISL_11900016	17.88	Omicron	BA.1.1	+	+	TP
GS0861	EPI_ISL_11900017	18.89	Omicron	BA.2	+	+	TP
GS0862	EPI_ISL_11900018	21.14	Omicron	BA.1.1	+	+	TP

Table II. Continued.

			Sequencing results[d]		RT-LAMP results[e]		
Sample[a]	GISAID ID[b]	Ct[c]	Variant	Pango lineage	Replicate 1	Replicate 2	Outcome[f]
GS0863	EPI_ISL_11900019	19.58	Omicron	BA.2	+	+	TP
GS0864	EPI_ISL_11900020	15.86	Omicron	BA.2	+	+	TP
GS0865	EPI_ISL_11900021	17.38	Omicron	BA.2	+	+	TP
GS0868	EPI_ISL_11900022	19.29	Omicron	BA.1	+	+	TP
GS0871	EPI_ISL_11900023	29.00	Omicron	BA.1.1	+	+	TP
GS0877	EPI_ISL_11900024	20.66	Omicron	BA.2	+	+	TP
GS0881	EPI_ISL_11900025	25.71	Omicron	BA.1.1	+	+	TP
GS0882	EPI_ISL_11900026	25.84	Omicron	BA.1.1	+	+	TP
GS0883	EPI_ISL_11900027	23.1	Omicron	BA.1.1	+	+	TP
GS0884	EPI_ISL_11900028	21.13	Omicron	BA.1.1	+	+	TP
GS0887	EPI_ISL_11900029	27.01	Omicron	BA.1.1	+	+	TP
GS0891	EPI_ISL_11900030	26.48	Omicron	BA.1.1	+	+	TP
GS0892	EPI_ISL_11900031	18.46	Omicron	BA.1.1	+	+	TP
GS0898	EPI_ISL_11900032	22.72	Omicron	BA.1	+	+	TP
GS0917	EPI_ISL_11900044	22.27	Omicron	BA.2	+	+	TP
GS0918	EPI_ISL_11900045	17.68	Omicron	BA.2	+	+	TP
GS0919	EPI_ISL_11900046	20.45	Omicron	BA.1.1	+	+	TP
GS0920	EPI_ISL_11900047	21.72	Omicron	BA.1.1	+	+	TP
GS0921	EPI_ISL_11900048	17.53	Omicron	BA.1.1	+	+	TP
GS0924	EPI_ISL_11900050	12.6	Omicron	BA.1.1	+	+	TP
GS1098	EPI_ISL_13203076	23.65	Omicron	BA.2	-	-	FN
GS1099	EPI_ISL_13203077	24.16	Omicron	BA.1.1	+	+	TP
GS1100	EPI_ISL_13203078	24.09	Omicron	BA.1.1	+	+	TP
GS1101	EPI_ISL_13203079	18.37	Omicron	BA.2	+	+	TP
GS1102	EPI_ISL_13203080	25.02	Omicron	BA.1.1	-	-	FN
GS1103	EPI_ISL_13203081	19.20	Omicron	BA.1.1	+	+	TP
GS1104	EPI_ISL_13203082	16.13	Omicron	BA.1.1	-	-	FN
GS1105	EPI_ISL_13203083	15.91	Omicron	BA.1.1	+	+	TP
GS1106	EPI_ISL_13203084	20.40	Omicron	BA.1.1	+	+	TP
GS1107	EPI_ISL_13203085	24.21	Omicron	BA.1.1	-	-	FN
GS1108	EPI_ISL_13203086	20.20	Omicron	BA.1.1	+	+	TP
GS1109	EPI_ISL_13203087	25.95	Omicron	BA.1.1	+	+	TP
GS1110	EPI_ISL_13203088	25.47	Omicron	BA.1.1	+	+	TP
GS1111	EPI_ISL_13202985	25.14	Omicron	BA.1.14	+	+	TP
GS1112	EPI_ISL_13202986	20.50	Omicron	BA.2.10	-	+	FN
GS1113	EPI_ISL_13202987	18.70	Omicron	BA.1.1	+	+	TP
GS1116	EPI_ISL_13202990	19.46	Omicron	BA.1.1	-	-	FN
GS1117	EPI_ISL_13202991	26.53	Omicron	BA.1	+	+	TP
GS1128	EPI_ISL_13202999	18.16	Omicron	BA.1.15	-	+	FN
GS1118	EPI_ISL_13202992	25.88	Omicron	BA.1.1	+	+	TP
GS1119	EPI_ISL_13202993	25.17	Omicron	BA.1.1	+	+	TP
GS1121	EPI_ISL_13202994	17.92	Omicron	BA.1.1	+	+	TP
GS1122	EPI_ISL_13202995	25.50	Omicron	BA.1.1	+	+	TP
GS0677	EPI_ISL_11899931	16.11	Delta	AY.59	-	-	TN
GS0679	EPI_ISL_11899933	15.74	Delta	AY.59	-	-	TN
GS0681	EPI_ISL_11899934	20.39	Delta	AY.79	-	-	TN
GS0682	EPI_ISL_11899935	24.49	Delta	AY.79	-	-	TN
GS0683	EPI_ISL_11899936	25.45	Delta	AY.79	-	-	TN
GS0685	EPI_ISL_11899938	20.08	Delta	AY.75	-	-	TN
GS0686	EPI_ISL_11899939	20.25	Delta	AY.79	-	-	TN

Table II. Continued.

Sample[a]	GISAID ID[b]	Ct[c]	Sequencing results[d]		RT-LAMP results[e]		Outcome[f]
			Variant	Pango lineage	Replicate 1	Replicate 2	
GS0688	EPI_ISL_11899940	22.91	Delta	AY.59	-	-	TN
GS0689	EPI_ISL_11899941	24.39	Delta	AY.59	-	-	TN
GS0690	EPI_ISL_11899942	27.62	Delta	AY.59	-	-	TN
GS0691	EPI_ISL_11899943	24.93	Delta	AY.59	-	-	TN
GS0694	EPI_ISL_11899945	21.38	Delta	AY.23	-	-	TN
GS0695	EPI_ISL_11899946	23.02	Delta	AY.79	-	-	TN
GS0696	EPI_ISL_11899947	21.25	Delta	AY.59	-	-	TN
GS0697	EPI_ISL_11899948	17.66	Delta	AY.23	-	-	TN
GS0698	EPI_ISL_11899949	20.02	Delta	AY.59	-	-	TN
M001	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M002	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M003	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M004	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M005	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M006	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M007	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M008	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M009	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M010	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M011	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M012	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M013	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M014	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M015	NA	ND	Non-COVID-19 samples	ND	-	-	TN
M016	NA	ND	Non-COVID-19 samples	ND	-	-	TN
XBB							
GU0399	EPI_ISL_15775985	25.72	XBB	XBB.1	+	+	TP
GU0411	EPI_ISL_15775982	19.37	XBB	XBB.1	+	+	TP
GU0412	EPI_ISL_15775981	18.82	XBB	XBB.1	+	+	TP
GU0419	NA	21.11	XBB	XBB.1	+	+	TP
GU0420	NA	26.70	XBB	XBB.1	+	+	TP
GU0425	NA	16.03	XBB	XBB.1	+	+	TP
GU0426	NA	19.35	XBB	XBB.1	+	+	TP
GU0428	NA	22.97	XBB	XBB.1	+	+	TP

Table II. Continued.

Sample[a]	GISAID ID[b]	Ct[c]	Sequencing results[d]		RT-LAMP results[e]		Outcome[f]
			Variant	Pango lineage	Replicate 1	Replicate 2	
GU0429	NA	23.52	XBB	XBB.1	+	+	TP
GU0431	NA	20.21	XBB	XBB.1	+	+	TP
GU0434	NA	21.10	XBB	XBB.2	+	+	TP
GU0435	NA	26.45	XBB	XBB.1	+	+	TP
GU0436	NA	20.03	XBB	XBB.1	+	+	TP
GU0438	NA	27.15	XBB	XBB.1	+	+	TP
GU0439	NA	20.12	XBB	XBB.1	+	+	TP
GU0444	NA	20.24	XBB	XBB.1	-	-	FN
GU0445	NA	17.70	XBB	XBB.1	+	+	TP
GU0461	NA	17.54	XBB	XBB.1	+	+	TP
GU0484	NA	20.63	XBB	XBB.2	+	+	TP
GU0486	NA	21.50	XBB	XBB.1	+	+	TP
GU0488	NA	18.33	XBB	XBB.1	+	+	TP
GU0489	NA	16.68	XBB	XBB.1	+	+	TP
GU0502	NA	18.65	XBB	XBB.1	+	+	TP
GU0503	NA	19.48	XBB	XBB.1	+	+	TP
GU0504	NA	23.40	XBB	XBB.1	+	+	TP
GU0507	NA	22.03	XBB	XBB.1	+	+	TP
GU0511	NA	22.29	XBB	XBB.1.1	+	+	TP
GU0518	NA	19.06	XBB	XBB.1	+	+	TP
GU0540	NA	17.91	XBB	XBB.1	+	+	TP
GU0547	NA	15.49	XBB	XBB.1.1	+	+	TP
GU0557	NA	18.30	XBB	XBB	+	+	TP
GU0566	NA	17.15	XBB	XBB.1	+	+	TP
GU0568	NA	20.48	XBB	XBB.1	+	+	TP
GU0494	NA	15.81	XBB	XBB.1	+	+	TP
GU0496	NA	20.46	XBB	XBB	+	+	TP
GU0601	EPI_ISL_16609619	23.12	XBB	XBB.1	+	+	TP
GU0609	EPI_ISL_16609624	15.62	XBB	XBB.1	+	+	TP
GU0610	EPI_ISL_16609625	25.16	XBB	XBB.1	+	+	TP
GU0611	EPI_ISL_16609626	20.24	XBB	XBB.1	-	-	FN
GU0612	EPI_ISL_16609627	19.19	XBB	XBB.1	+	+	TP
GU0613	EPI_ISL_16609628	17.38	XBB	XBB	+	+	TP
GU0618	EPI_ISL_16609633	21.20	XBB	XBB	+	+	TP
GU0620	EPI_ISL_16609634	23.17	XBB	XBB.1	+	+	TP
GU0625	EPI_ISL_16609639	21.42	XBB	XBB.1	+	+	TP
GU0626	EPI_ISL_16609640	23.31	XBB	XBB.1.1	+	+	TP
GU0632	EPI_ISL_16609646	23.10	XBB	XBB.1	+	+	TP
GU0635	EPI_ISL_16609649	25.97	XBB	XBB.1	+	+	TP
GU0636	EPI_ISL_16609650	26.25	XBB	XBB.1	+	+	TP
GU0639	EPI_ISL_16609653	20.27	XBB	XBB.1	-	-	FN
GU0641	EPI_ISL_16609655	22.62	XBB	XBB.1	+	+	TP
GU0648	NA	27.21	XBB	XBB.2	+	+	TP
GU0652	EPI_ISL_16609658	20.12	XBB	XBB.1	-	-	FN
GU0656	EPI_ISL_16609662	17.80	XBB	XBB.1.1	+	+	TP
GU0659	EPI_ISL_16609664	15.80	XBB	XBB.1	-	-	FN
GU0660	EPI_ISL_16609665	24.32	XBB	XBB.1	+	+	TP
GU0661	EPI_ISL_16609666	24.93	XBB	XBB.1	+	+	TP
GU0590	NA	24.20	XBB	XBB.1	+	+	TP
GU0585	NA	21.50	XBB	XBB.1	+	+	TP

Table II. Continued.

Sample[a]	GISAID ID[b]	Ct[c]	Sequencing results[d]		RT-LAMP results[e]		Outcome[f]
			Variant	Pango lineage	Replicate 1	Replicate 2	
GU0583	NA	22.06	XBB	XBB.1	+	+	TP
GU0570	NA	26.10	XBB	XBB.1	+	+	TP
GU0571	NA	25.92	XBB	XBB.1	+	+	TP
GU0137	EPI_ISL_13651378	17.24	Omicron	BA.2	-	-	TN
GU0136	EPI_ISL_13651377	17.13	Omicron	BA.1.1	-	-	TN
GU0135	EPI_ISL_13651376	26.75	Omicron	BA.2	-	-	TN
GU0133	EPI_ISL_13651374	19.09	Omicron	BA.1.1	-	-	TN
GU0134	EPI_ISL_13651375	26.51	Omicron	BA.2.10	-	-	TN
GU0131	EPI_ISL_13651372	20.32	Omicron	BA.2.3	-	-	TN
GU0132	EPI_ISL_13651373	24.9	Omicron	BA.1.1	-	-	TN
GU0130	EPI_ISL_13651371	23.61	Omicron	BA.2.3	-	-	TN
GU0196	EPI_ISL_13203014	16.66	Omicron	BA.2	-	-	TN
GU0197	EPI_ISL_13203015	21.27	Omicron	BA.2	-	-	TN
GU0195	EPI_ISL_13203013	15.06	Omicron	BA.1.1	-	-	TN
GU0194	EPI_ISL_13203012	13.17	Omicron	BA.2	-	-	TN
GU0193	EPI_ISL_13203011	14.28	Omicron	BA.2	-	-	TN
GU0192	EPI_ISL_14217738	23.17	Omicron	BA.2	-	-	TN
GU0186	EPI_ISL_14217732	17.12	Omicron	BA.2	-	-	TN
GU0181	EPI_ISL_14217727	24.85	Omicron	BA.2	-	-	TN
GU0179	EPI_ISL_12277176	20.86	Omicron	BA.2	-	-	TN
GU0180	EPI_ISL_14217726	19.10	Omicron	BA.1.1	-	-	TN
GU0178	EPI_ISL_12277175	17.42	Omicron	BA.2.9	-	-	TN
GU0189	EPI_ISL_14217735	17.27	Omicron	BA.2.3	-	-	TN
GU0188	EPI_ISL_14217734	23.90	Omicron	BA.2.3	-	-	TN
GU0187	EPI_ISL_14217733	21.01	Omicron	BA.2.40.1	-	-	TN
GU0185	EPI_ISL_14217731	24.48	Omicron	BA.2	-	-	TN
GU0184	EPI_ISL_14217730	25.79	Omicron	BA.2.3	-	-	TN
GU0183	EPI_ISL_14217729	19.69	Omicron	BA.2	-	-	TN
GU0042	EPI_ISL_14217725	25.22	Omicron	BA.2.3	-	-	TN
GU0041	EPI_ISL_14217724	20.13	Omicron	BA.2.3	-	-	TN
GS1123	EPI_ISL_13202996	17.25	Omicron	BA.1.1	-	-	TN
GS1125	EPI_ISL_13202997	26.27	Omicron	BA.1.1	-	-	TN
GS1126	EPI_ISL_13202998	17.22	Omicron	BA.1.1	-	-	TN
GS1129	EPI_ISL_13203000	18.64	Omicron	BA.1.1	-	-	TN
GS1130	EPI_ISL_13203001	17.11	Omicron	BA.1.1	-	-	TN
GS1118	EPI_ISL_13202992	26.00	Omicron	BA.1.1	-	-	TN
GS1131	EPI_ISL_13203002	25.89	Omicron	BA.1.1	-	-	TN
GS1122	EPI_ISL_13202995	18.68	Omicron	BA.1.1	-	-	TN
GS0868	EPI_ISL_11900022	16.65	Omicron	BA.1	-	-	TN
GS1119	EPI_ISL_13202993	19.23	Omicron	BA.1.1	-	-	TN
GS1121	EPI_ISL_13202994	16.06	Omicron	BA.1.1	-	-	TN
GS1128	EPI_ISL_13202999	24.34	Omicron	BA.1.15	-	-	TN
GS1117	EPI_ISL_13202991	23.68	Omicron	BA.1	-	-	TN
GS1112	EPI_ISL_13202986	26.02	Omicron	BA.2.10	-	-	TN
GS1113	EPI_ISL_13202987	20.47	Omicron	BA.1.1	-	-	TN
GS1111	EPI_ISL_13202985	23.80	Omicron	BA.1.14	-	-	TN
GS1008	EPI_ISL_11976209	22.78	Omicron	BA.1.1	-	+	FP
GS1022	EPI_ISL_12277129	24.54	Omicron	BA.1.1	+	+	FP
GS1015	EPI_ISL_11976215	18.12	Omicron	BA.1.1	+	+	FP
GS1018	EPI_ISL_11976216	25.06	Omicron	BA.1.1	+	+	FP

Table II. Continued.

Sample[a]	GISAID ID[b]	Ct[c]	Sequencing results[d]		RT-LAMP results[e]		Outcome[f]
			Variant	Pango lineage	Replicate 1	Replicate 2	
GS1020	EPI_ISL_11976217	21.37	Omicron	BA.1.1	+	+	FP
GS1021	EPI_ISL_11976218	25.20	Omicron	BA.1.1	+	+	FP
GS1024	EPI_ISL_12277130	25.74	Omicron	BA.1.1	+	+	FP
GS1028	EPI_ISL_12277134	24.14	Omicron	BA.1.1	+	+	FP
GS1029	EPI_ISL_12277135	18.72	Omicron	BA.2.10	+	+	FP
GS0987	EPI_ISL_11976196	22.39	Omicron	BA.1	+	+	FP
GS1030	EPI_ISL_12277136	22.60	Omicron	BA.2.10	+	+	FP
GS1031	EPI_ISL_12277137	23.79	Omicron	BA.2.10	+	+	FP
GS1032	EPI_ISL_12277138	25.53	Omicron	BA.2.10	+	+	FP
GS1033	EPI_ISL_12277139	17.87	Omicron	BA.2.10	-	-	TN
GS1042	EPI_ISL_12277146	18.87	Omicron	BA.2.10	+	-	FP
GS1041	EPI_ISL_12277145	18.52	Omicron	BA.2.10	+	+	FP
GS1039	EPI_ISL_12277143	23.28	Omicron	BA.2.10	+	+	FP
GS1037	EPI_ISL_12277142	22.93	Omicron	BA.2.10	+	+	FP
GS0988	EPI_ISL_11976197	20.09	Omicron	BA.1	-	+	FP
GS1036	EPI_ISL12277141	20.00	Omicron	BA.2.10	+	+	FP

[a]Sample identification code based on the list provided by the Advanced Genomic and Bioinformatic Laboratory; [b]GISAID ID refers to unique identifier generated by GISAID for each sequence deposition; [c]the Ct column corresponds to the quantification cycle of the sample positive for SARS-CoV-2 using the 2019nCoV primer kit; [d]results of sequencing obtained in the GISAID EpiCoV platform; [e]results of RT-LAMP assay in duplicate; [f]outcome of the RT-LAMP assay. Ct, cycle threshold; -, negative result (no color change); +, positive result (color change); NA, not applicable (NA for COVID-19 samples refers to an entry rejected by GISAID due to poor quality); ND, not detected; TP, true positive; TN, true negative; FP, false positive; FN, false negative; RT-LAMP, reverse-transcription loop-mediated isothermal amplification; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table III. Summary of the RT-LAMP clinical results.

SARS-CoV-2 variant	Performance			No. of samples		
	Sensitivity (%)	Specificity (%)	Accuracy (%)	Positive	Negative	Total
Delta	100	43.75	71.57	47	48	95
Omicron	89.47	100	92.18	95	33	128
XBB	91.80	69.84	80.64	61	63	124

All Delta, Omicron and XBB were confirmed positive by sequencing.

turnaround time, with results available in 30 min to 1 h. This is a notable improvement over RT-qPCR, which typically takes at least 2 h, and NGS, which can take up to 1 or 2 days. These attributes render RT-LAMP a promising tool for rapid diagnosis and VOC surveillance.

With vaccinations rising globally, keeping track of new variants is crucial. This helps policymakers make informed decisions and prevent outbreaks from highly transmissible or antibody-evading variants, such as Omicron. The present study highlights how a special type of RT-LAMP test, designed to target specific lineages, can be a valuable tool. It allows researchers to estimate how common these lineages are within a population during a specific week. This test could be particularly useful in areas where sequencing resources are limited or expensive. It can act as a preliminary screening method before resorting to more in-depth sequencing techniques.

There are two main ways to improve these assays: Exploring enzymes better suited for displacing existing strands and creating cost-effective, in-house reagents. In fact, a previous study was conducted to evaluate the usefulness of freeze-dried (lyophilized) RT-LAMP reagents as at-home self-testing kit (5). The authors of that study demonstrated that lyophilized RT-LAMP reactions had fewer false positives compared to those using liquid reagents. This technique offers several advantages: An extended shelf life,

room-temperature storage and the elimination of cold-chain shipping costs (5).

While RT-LAMP offers significant advantages for SARS-CoV-2 detection, it is essential to acknowledge its limitations. One notable concern is the risk of false positive results, which can arise from non-specific amplification, especially when using colorimetric detection (15). Suboptimal primer design can also lead to misamplification (16). Additionally, sample quality can impact assay performance. In particular, the presence of inhibitors in crude samples such as saliva, blood, and urine can affect the accuracy of the test (12). Some studies have found that RT-LAMP becomes less reliable for samples with high cycle quantification values (Cq) on RT-qPCR. Moreover, visual interpretation of colorimetric results can introduce user errors, and extended storage at elevated temperatures can reduce the colour contrast, increasing misinterpretations (5). Despite these limitations, the ongoing optimization of RT-LAMP assays aims to reduce these issues. For example, the use of five primers instead of six can reduce the false positive rate (15).

Integrating RT-LAMP into current COVID-19 testing strategies, particularly in low-resource settings, requires a strategic approach. Due to its low cost and simplicity, RT-LAMP can be used as a screening tool to identify potentially positive cases. Positive results can then be confirmed using a more accurate test like RT-qPCR, if resources allow. The rapid turn-around time of RT-LAMP makes it valuable for mass surveillance, and this is particularly important for identifying the virus earlier in the transmissible phase. RT-LAMP assays that utilize a simple workflow and minimal equipment are ideal for use in decentralized settings, such as local pharmacies, mobile labs or in the home. In these situations, a technician or the patient themselves would take the sample using a swab and then return the sample for testing. Furthermore, the capacity of RT-LAMP assays to detect variants using specific primers makes it useful for monitoring emerging variants. Overall, the trade-offs between speed, cost and accuracy should be considered when deploying RT-LAMP as a complementary tool to existing methods, particularly in low resource settings.

In conclusion, these novel SARS-CoV-2 variant detection methods are innovative. At under $5 per test (excluding labor and processing), they are significantly more cost-effective than existing options. In addition, they can be easily scaled up or down to fit the needs of any setting, from small workplaces with a handful of employees to schools with a few thousand students. The simple equipment needed and the low cost render them ideal for areas with limited resources, which have often been hit hardest by the pandemic.

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Availability of data and materials

The DNA sequencing data for the SARS-CoV-2 variants tested in the present study may be found in the GISAID repository at www.gisaid.org using the respective GISAID IDs outlined in Table II. The user may view and/or download information using the respective GISAID IDs at the GISAID database. First, the user needs to register an account and log in to GISAID at www.gisaid.org. Upon logging in, the user can click on the 'EpiCoV' database and enter the EPI_ISL ID into the search tool. Information regarding the entry will be available in a new window.

Authors' contributions

All authors (KOL, NMI, AA and NAJ) contributed to the conception and design of the study. Genome analysis and alignment was performed by AA. The SARS-CoV-2 RNA samples were prepared by NMI and NAJ. The LAMP primer design and LAMP assay were performed by NMI and KOL. NMI and KOL confirm the authenticity of all the raw data. The first draft of the manuscript was written by KOL and all authors commented on previous versions of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The National Institutes of Biotechnology Malaysia confirmed that no ethics approval or patient consent were required for the present study due to the following reasons: The study was conducted as part of a government-funded initiative during the COVID-19 pandemic, which prioritized rapid response to public health needs. The samples used for the project were provided by government hospitals specifically for surveillance and sequencing purposes, in alignment with national public health objectives. Under such circumstances, the project fell within the scope of public health surveillance, which is often exempt from individual consent requirements. Additionally, the study adhered to strict data protection protocols, ensuring that all samples were anonymized and used solely for the purpose of developing diagnostic methods to combat the pandemic.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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