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Letter to the Editor

Adaptability of single-nucleotide polymorphism-polymerase chain reaction (SNP-PCR) for subtyping SARS-CoV-2 and a new SNP-PCR for XBB, XBB.1.5, and B.Q.1/B.Q.1.1

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To the Editor,

Single-nucleotide polymorphism (SNP)-PCR has been proposed as a rapid, reliable and complementary method to whole-genome sequencing (WGS) for SARS-CoV-2 surveillance [1,2]. The advantages of SNP-PCR include the ability of front-line clinical laboratories to easily implement the method without additional instrumentation or bioinformatics, flexibility to edit or update the SNP-PCR as new SARS-CoV-2 variants of concern (VOC) emerge, and rapid turnaround time when needed for clinical management (e.g. determining the effectiveness of monoclonal antibody therapy or investigating prolonged infection versus re-infection). From a laboratory workflow and utilization perspective, SNP-PCR also allows WGS resources to be best reserved for atypical or newly emerging strains, whereas the SNP-PCR rapidly identifies the most common and predominant VOC. Currently, public health experts are calling for prompt testing to identify new cases of the latest emerging variants: XBB, XBB.1.5 and B.Q.1/B.Q.1.1.

Our clinical virology laboratory has performed SNP-PCR for SARS-CoV-2 subtyping on positive clinical samples weekly since January 2021 [3], using various combinations of the commercially available VirSNiP assays (TIB Molbiol, Germany) and end-point hydrolysis probe PCR, validated with WGS (Illumina MiSeq). For all samples undergoing SNP-PCR, 500 μL underwent nucleic acid extraction using MagNA Pure 96 (Roche Molecular Systems Inc., USA) into 50 μL of eluate, followed by PCR amplification according to the manufacturer's protocols using LightCycler 480 (Roche). Our SNP-PCR algorithm has evolved many times over the course of the pandemic to incorporate new mutations reflecting the latest VOC (Table 1). As readers may currently seek to implement testing protocols in their own laboratories, we describe our previous SNP-PCR algorithms for Omicron variants and the design of a new SNP-PCR to differentiate XBB, XBB.1.5 and B.Q.1/B.Q.1.1 in a single well, which has not been previously described.

In September 2022, a single-well multiplex PCR was designed to differentiate Omicron sublineages BA.1, BA.2, BA.4 and BA.5. VirSNiP matrix D3N/G, detected using the manufacturer's protocol for SimpleProbe melting curve analysis, was combined with end-point PCR for spike L452R and del69/70 (Table 1), allowing for detection of each molecular target in the same PCR reaction. During the validation, the per cent agreement of SNP-PCR with WGS was excellent: 20 of 20 (100%) BA.1 samples, 26 of 26 (100%) BA.2 samples, 7 of 7 (100%) BA.4 samples and 20 of 20 (100%) BA.5 samples. An additional two samples had atypical/non-definitive results by SNP-PCR that required WGS for complete analysis: these were found to have rare mutations (one BA.2 sample with mutation L452R, and one BA.5 sample with matrix mutation T7I, altering the D3N/G probe melting temperature). Notably, an additional eight samples (three BA.1, two BA.2 and three BA.4) could not be successfully analysed by WGS because of high cycle threshold values (median 32.05) but were able to be typed by SNP-PCR.

BA.5 was the predominant Omicron sublineage among our clinical samples from September 2022 to February 2023. By early February 2023, we observed an increase in the proportion of SARS-CoV-2 samples typing as BA.2. In March 2023, we implemented an updated SNP-PCR algorithm by combining VirSNiP F486S/P/V (SimpleProbe melting curve analysis) with end-point PCR for spike del69/

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Table 1
Evolution of the SARS-CoV-2 SNP-PCR algorithm in a clinical virology laboratory from January 2021 to March 2023

January 2021: 'Alpha, Beta, Gamma'				
Targets:				
N501Y	Detected	Detected	Detected	
del69/70	Detected	Not detected	Not detected	
VirSNP K417N/T	Not detected	K417N	K417T	
SARS-CoV-2 variant detected	B.1.1.7 (Alpha)	B.1.351 (Beta)	P.1 (Gamma)	
June 2021: 'Alpha, Beta, Gamma, Delta'				
To above algorithm, added the following targets in a new PCR:				
P681R	Detected	Detected		
VirSNP E484K/Q	E484Q	Not detected		
SARS-CoV-2 variant detected	B.1.617.1/B.1.617.3	B.1.617.2 (Delta)		
November 2021: 'Omicron'				
Confirmed the above existing algorithm could detect Omicron:				
N501Y	Detected			
del69/70	Detected			
VirSNP K417N/T	K417N			
SARS-CoV-2 variant detected	B.1.1.529 (Omicron)			
February 2022: 'Delta vs. Omicron'				
Completely revised the algorithm with new targets:				
VirSNP S371L/S373P	Not detected	Detected	Detected	
P681R	Detected	Not detected	Not detected	
del69/70	Not detected	Detected	Not detected	
SARS-CoV-2 variant detected	B.1.617.2 (Delta)	BA.1 (Omicron)	BA.2 (Omicron)	
September 2022: 'Omicron Variants'[a]				
Completely revised the algorithm with new targets:				
VirSNP Matrix D3N/G	D3G	D3N	D3 wildtype	D3 wildtype
L452R	Not detected	Detected	Detected	Not detected
del69/70	Detected	Detected	Detected	Not detected
SARS-CoV-2 variant detected	BA.1 (Omicron)	BA.5 (Omicron)	BA.4 (Omicron)	BA.2 (Omicron)[c]
March 2023: 'XBB, XBB.1.5, B.Q.1/B.Q.1.1'[b]				
Completely revised the algorithm with new targets:				
VirSNP F486S/P/V	F486S (55.5°C)	F486P (65°C)	F486V (50°C)	F486V (50°C)
del69/70	Not detected	Not detected	Detected	Detected
K444-Probe for K444T	V445P/G446S detected	V445P/G446S detected	K444T detected	K444 wildtype detected
Probe for K444 wildtype				
Probe for V445P/G446S				
SARS-CoV-2 variant detected	XBB	XBB.1.5 (Kraken)	B.Q.1/B.Q.1.1	BA.5 (Omicron), not B.Q.1/B.Q.1.1

SNP, single-nucleotide polymorphism.

[a] Primer and probe sequences used for the Omicron SNP-PCR assay: del6970F TCA ACT CAG GAC TTG TTC TTA C, del6970R TGG TAG GAC AGG GTT ATC AAA C, WT6970 HEX - TGC TAT + ACA TG + T CTC TGG GAC CA -IABkFQ (dye HEX580), del6970 TEX615 - TGC TAT + CTC TG + G GAC CAA TG - IAbRQSp (dye TEX615), 452F GCT GCG TTA TAG CTT GGA, 452R CGG CCT GAT AGA TTT CAG T, 452RP LC640 - TA ATT ATA ATT AC + C + T + GT ATA GAT TGT T-BHQ-2 (dye LC640), 452LP Cy5-TA ATT ATA ATT AC + C + G + GT ATA GAT TGT T-BHQ-2 (dye Cy5 660), VirSNP D3N/G (TIB Molbiol, cat# 53-0840-96).

[b] Primer and probe sequences used for the XBB, XBB.1.5, B.Q.1/B.Q.1.1 SNP-PCR assay: 444F AGG CTG CGT TAT AGC TTG, 444R CAA TCT ATA CAG GTA ATT ATA ATT ACC AC, 444BQ1 LC640 - TGA TTC T + A + C + GGT TGG TG-BHQ2 (dye LC640), 444WT Cyan500 - TGA TTC T + A + A + GGT TGG TGG-BHQ1 (dye Cyan500), 444XBB Cy5 - CAAGCTTGATTCTA + AGCCTAGTGGTAA-BHQ2 (dye Cy5 660), VirSNP F486 (TIB Molbiol, cat# 53-0852-96), del69/70 as above.

[c] Wildtype SARS-CoV-2 and Alpha, Beta, Gamma, Delta VOC would also have these mutations detected, but not currently in circulation.

70 and K444T, with multiple end-point hydrolysis probes (Table 1). Prospective evaluation during March 2023 demonstrated high per cent agreement with WGS: 26 of 26 (100%) B.Q.1/B.Q.1.1 samples, 1 of 1 (100%) XBB sample and 33 of 38 (86.8%) XBB.1.5 samples. Of the five samples with discordant WGS results, all were strains with identical spike target mutations as XBB.1.5: two were XBB.1.9.1, two were XBB.2.3 (with one additional spike mutation P521S) and one was XBB.1.16 (with additional spike mutations E180V and K478R). An additional two clinical samples had non-definitive SNP-PCR results, one with a unique SimpleProbe melting temperature for F486S (51°C). WGS confirmed that these were uncommon strains outside of our algorithm (XBF and CH.1.1.1). We observed the prevalence of XBB.1.5 among our clinical samples to increase from 35% in March 2023 to nearly 100% by early April 2023.

Our experience demonstrates that SNP-PCR is an accurate, flexible and easily scalable method for SARS-CoV-2 subtyping. Workflow is further optimized by the design of single-well multiplex PCR. In particular, our current SNP-PCR assay for XBB, XBB.1.5, and B.Q.1/B.Q.1.1 successfully differentiates a remarkably high number of SNPs in a single well (seven in total: F486V/S/P, del69/70, K444T, V445P and G446S). Furthermore, samples with atypical/non-definitive SNP-PCR results can be rapidly identified and prioritized for WGS. Recently, there has been detection in over 25 countries of the newly emerging SARS-CoV-2 variant XBB.1.16, closely related to XBB.1.5 but carrying additional spike mutations [4,5]. It would be straightforward to add an additional end-point PCR or potentially replace our current K444T target with one of these new mutations to focus on differentiation of XBB.1.16, because we have already identified one such strain among our clinical samples. As new variants of SARS-CoV-2 continue to emerge and laboratory WGS resources are challenged, SNP-PCR subtyping may continue to evolve and play a useful role in SARS-CoV-2 surveillance and clinical management.

Transparency declaration

Conflict of interest

The authors declare that they have no conflicts of interest.

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