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Detection of SARS-CoV-2 VOC-Omicron using commercial sample-to-answer real-time RT-PCR platforms and melting curve-based SNP assays

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ABSTRACT

Objectives: The World Health Organization (WHO) had designated the SARS-CoV-2 lineage B.1.1.529 as the new Variant of Concern Omicron (VOC-Omicron) on 26th November 2021[1]. Real-time reverse transcription polymerase chain reaction (RT-PCR), single nucleotide polymorphisms (SNP) and whole genome sequencing (WGS) tests were widely employed to detect SARS-CoV-2 and its variant. Yet, the SARS-CoV-2 Omicron detection performance of commercial real-time RT-PCR platforms and SARS-CoV-2 spike SNP assays remain to be elucidated.
Methods: In the first part of this study, we evaluated the VOC-Omicron detection performance of three commercial RT-PCR sample-to-answer platforms i.e. Roche cobas® 6800/8800, Roche cobas® Liat®, and Cepheid GeneXpert® systems. The detection performances were compared to one commercial conventional real-time RT-PCR assay (TIB MOLBIOL LightMix Modular SARS and Wuhan CoV E-gene) and one in-house real-time RT-PCR assay targeting RNA-dependent RNA polymerase (RdRP) gene of SARS-CoV-2 in the WHO COVID-19 Reference Laboratory at Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health, The Government of the Hong Kong Special Administrative Region. In the second part of this study, we evaluated the SNP detection performance of four TIB MOLBIOL melting curve-based assays (1. Spike S371L/S373P, 2. Spike E484A, 3. Spike E484K and 4. Spike N501Y) in clinical samples obtained from hospitalized COVID-19 patients in Hong Kong. The SNP results were compared to whole genome sequences generated by Illumina platform.
Results: The VOC-Omicron detection limits of three commercial sample-to-answer assays were tested to be ≤ 2.35 Log_{10} dC/ml. The detection performances of the sample-to-answer platforms were comparable to the two tested conventional real-time RT-PCR assays. The test sensitivities of TIB MOLBIOL VirSNiP SARS-CoV-2 Spike S371L/S373P assay and the Spike E484A assays were 100% and 96.6% respectively and the test specificities of both assays were 100%. An aberrant melting peak at Tm 42-44°C was observed when the specimens with Omicron variant were tested with the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike E484K assay. Notably, the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike N501Y assay failed to detect the spike N501Y mutation of Omicron variant in the tested specimens.
Conclusions: The SARS-CoV-2 detection sensitivity of three commercial platforms, Roche cobas® 6800/8800, Roche cobas® Liat®, and Cepheid GeneXpert® systems were shown not to be impacted by the large number of mutations of VOC-Omicron. Also, the signature mutations i.e. Spike S371L/Spike S373P and Spike E484A in VOC-Omicron were correctly identified by the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike S371L/S373P and VirSNiP SARS-CoV-2 Spike E484A assays. Unexpected findings including a shifted melting peak or absence of amplification curve/melting peak were observed when specimens with Omicron variant were tested with the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike E484K assay and Spike N501Y assay, suggesting a potential alert for Omicron variant, prior confirmation by whole genome sequencing.

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1. Introduction

The World Health Organization had designated the SARS-CoV-2 lineage B.1.1.529 as the new Variant of Concern Omicron (VOC-Omicron) on 26th November 2021 [1]. The first whole genome sequence of VOC-Omicron available on GISAID (published by our group on 22 Nov 2021; accession ID: EPI_ISL_6590782) possesses 8, 2, 34, 1, 3, and 3 mutations in ORF1a, ORF1b, spike (S), envelope (E), membrane (M), and nucleocapsid (N) genes respectively. Subsequently, VOC-Omicron had split into three sub-lineages namely BA.1, BA.2 and BA.3 [2]. The VOC-Omicron sub-lineages contain unusually high number of novel mutation sites in the spike gene and have altogether formed a new monophyletic clade from other SARS-CoV-2 variants [3,4]. Although most SARS-CoV-2 real-time RT-PCR assays target conserved regions across the genome, it was indicated in the World Health Organization statement that the VOC-Omicron has led to spike gene dropout (i.e. spike gene target failure) in one widely used PCR test for SARS-CoV-2 detection [1]. In the same statement, the spike gene dropout was suggested to be used as marker for this variant, pending sequencing confirmation.

Real-time reverse transcription polymerase chain reaction (RT-PCR) is widely employed to detect SARS-CoV-2 in clinical specimens. Although high SARS-CoV-2 detection sensitivity of automated RT-PCR-based systems such as GeneXpert® system (Cepheid, USA), cobas® 6800/8800 systems (Roche, Switzerland) and cobas® Liat® (Roche) were reported [5,6], it was previously reported that the cobas SARS-CoV-2 E gene RT-PCR and the Cepheid Xpert Xpress SARS-CoV-2 N gene RT-PCR were adversely affected by mutations in the SARS-CoV-2 E gene (C26340T) and N gene (C29200T) respectively [7–9]. Of note, the impact of Omicron variant on these commercial sample-to-answer platforms has not been assessed.

Post-amplification melting curve-based SNP screening assays are used as fast, cost-effective, and high-throughput approaches for SARS-CoV-2 variants screening and identification [10,11]. TIB MOLBIOL VirSNiP SARS-CoV-2 SNP assays (TIB MOL BIOL, Germany) had been widely used for SARS-CoV-2 VOC screening and surveillance [12,13]. Previous studies showed a >98% detection sensitivity of signature mutations, i.e. spike Spike N501Y in VOC-Alpha/ Beta and spike Spike E484K in VOC-Beta variant using the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike N501Y or E484K assays [13]. Yet, the signature mutation detection performance of TIB MOLBIOL VirSNiP SARS-CoV-2 SNP assays on Omicron variant remains to be elucidated.

In the current study, we first evaluated the VOC-Omicron detection performance of three sample-to-answer platforms. The detection performance were compared to TIB MOLBIOL LightMix Modular SARS and Wuhan CoV E-gene assay and one in-house SARS-CoV-2 real-time RT-PCR assay targeting RdRP gene. Secondly, we evaluated the SNP detection sensitivity and specificity of four TIB MOLBIOL VirSNiP SARS-CoV-2 Spike S371L/S373P, Spike E484A, Spike E484K and Spike N501Y assays. Clinical specimens with SARS-CoV-2 and variants including VOCs-Alpha, Beta, Delta and Omicron were tested.

2. Materials and methods

2.1. SARS-CoV-2 detection by commercial Sample-to-answer platforms

A serial dilution of a throat saliva sample (VM21045928) detected with Omicron variant and Qnostics SARS-CoV-2 Analytical Q Panel 01 (whole virus; RANDOX, UK) [14] were tested using three commercial assays, namely cobas® SARS-CoV-2 Test, cobas® SARS-CoV-2 & Influenza A/B Assay, and Xpert® Xpress SARS-CoV-2 on three sample-to-answer platforms (cobas® 6800/8800 Systems, cobas® Liat® System and GeneXpert® system respectively) according to the manufacturer's instructions.

2.2. SARS-CoV-2 detection by conventional real-time RT-PCR

Using EZ1 Virus Mini Kit v2.0 (QIAGEN, Germany), total nucleic acid was extracted from the serially diluted specimens (VM21045928) with Omicron variant and Qnostics SARS-CoV-2 Analytical Q Panel 01 on Qiagen EZ1 Advanced XL platform. 400µL of specimen was loaded into the cartridge according to manufacturer's instruction, and 120µL of eluate was obtained and stored at -70°C until use. Real-time RT-PCR using LightMix Modular SARS and Wuhan CoV E-gene detection assay (Cat. No. 53-0776-96) with LightCycler Multiplex RNA Virus Master was performed on LightCycler 480 II according to the manufacturer's instructions. In-house SARS-CoV-2 RT-PCR assay (targeting RdRP gene) developed by WHO COVID-19 Reference Laboratory at Public Health Laboratory Services Branch (PHLSB), Centre for Health Protection, Department of Health, The Government of the Hong Kong Special Administrative Region was also performed on LightCycler 480 II. Each 10µl reaction mixture contained 5 µl of template, 2µl NxtScript DNA Master, 0.05µl NxtScript Reverse Transcriptase, 0.06µl AptaTaq DNA Polymerase, 0.79µl nuclease free water, and a total of 2.1µl primer and probe mix **(listed in Supplementary Table 1)**. The thermocycling condition was 50°C for 10 minutes, 95°C for 30 seconds, followed by 40 cycles of 95°C for 10 seconds and 56°C for 30 seconds.

The SARS-CoV-2 viral concentration of Qnostics SARS-CoV-2 Analytical Q Panel 01 was quantified by the manufacturer using digital PCR [14]. The panel was included in this study for limit of detection (LoD) comparison among different testing platforms and establishment of a standard curve for SARS-CoV-2 quantification in the specimens.

2.3. SARS-CoV-2 spike SNP detection by TIB MOLBIOL VirSNiP assays

To assess the SNP detection performances of TIB MOLBIOL VirSNiP SARS-CoV-2 Spike E484A assay (Cat. No. 53-0829-96) and VirSNiP SARS-CoV-2 Spike S371L/S373P assay (Cat. No. 53-0827-96), 44 clinical specimens from 39 patients were tested. The Pangolin lineages of SARS-CoV-2 detected in the specimens were B.1.1.529 (VOC-Omicron sub-lineage BA.1, n = 29), B.1.1.7 (VOC-Alpha, n = 3), B.1.351 (VOC-Beta, n = 3), B.1.617.2 (VOC-Delta, n = 3), B.1.617.1 (Kappa, n = 3), A (n = 1), B.1.1.317 (n = 1) and B.1.466.2 (n = 1) respectively, as confirmed by whole genome sequencing.

To assess the SNP detection performances of TIB MOLBIOL VirSNiP SARS-CoV-2 Spike E484K assay (Cat. No. 53-0789-96) and VirSNiP SARS-CoV-2 Spike N501Y assay (Cat. No. 53-0780-96), 41 clinical specimens from 39 patients were tested. The Pangolin lineages of SARS-CoV-2 detected in the specimens were B.1.1.529 (VOC-Omicron sub-lineage BA.1, n = 22), B.1.1.7 (VOC-Alpha, n = 5), B.1.351 (VOC-Beta, n = 4), B.1.617.2 (VOC-Delta, n = 4), B.1.617.1 (Kappa, n = 3), A (n = 1), B.1.1.317 (n = 1) and B.1.466.2 (n = 1) respectively.

The TIB MOLBIOL VirSNiP assays were performed on LightCycler 480 II according to manufacturer's instruction. All four SNP detection assays differentiate SNPs by fluorescence-based post-amplification melting-curve analysis after thermocycling. The Spike S371L/S373P assay and Spike N501Y assay give amplification signals in FAM channel if Spike S371L/S373P and Spike N501Y mutations are detected.

2.4. Whole genome sequencing

The whole genome sequencing of SARS-CoV-2 was performed according to previous studies [15].

3. Results

3.1. VOC-Omicron detection performance on commercial Sample-to-answer and conventional real-time RT-PCR platforms

The limit of detection (LOD) of the three tested commercial sample-to-answer platforms in detecting VOC-Omicron was shown to be 2.35

Table 1
Comparison of detection limit of three commercial sample-to-answer platforms and the conventional real-time RT-PCR assays.

	PHLSB in-house assay	TIB MOLBIOL E-gene	Cobas 6800/8800 cobas® SARS-CoV-2 Test	GeneXpert Xpert® Xpress SARS-CoV-2	Liat cobas® SARS-CoV-2 & Influenza A/B Assay
	LoD* (Log_{10} dC/ml) [RT-PCR gene target(s): respective Cp/Ct values in duplicate except Liat]				
Omicron variant	2.35 [RdRP: 34.21 / 34.51]	2.35 [E: 34.37 / 34.77]	1.35 [Orf1ab: 37.41 / 36.13; E: ND / 37.03]	2.35 [E: 34.6 / 35.6; N2: 38.0 / 37.9]	1.35 [Orf1ab+N: 33.58 / 34.80]
Qnostics SARS-CoV-2 Analytical Q Panel 01	2 [RdRP: 35.45 / 36.24]	2 [E: 35.11 / 34.5]	1 ['Orf1ab: 36.43 / 36.84; E: 38.82 / 37.80]	2 [E: 35.3 / 36.8; N2: 38.6 / 38.4]	1# [Orf1ab+N: 35.12 / 36.75 / ND]

* The SARS-CoV-2 viral concentrations of tested specimens were quantified by a standard curve-based method established using the Qnostics SARS-CoV-2 Analytical Q Panel 01 (RANDOX, UK) and the PHLSB in-house SARS-CoV-2 RT-PCR assay; ND: Not Detected.
# Test in triplicate.

Table 2
Sensitivity and specificity of TIB MOLBIOL VirSNiP SARS-CoV-2 Spike SNP assays with reference to whole genome sequencing.

TIB MOLBIOL VirSNiP SARS-CoV-2 Spike S371L/S373P assay	**Samples tested**	**Melting-curve analysis**					**WGS analysis**				**Sensitivity**	**Specificity**
		S371L/S373P		**S371S/S373S**	**Ab Tm**	**No Tm**	**Omicron S371L/S373P**		**Others S371S/S373S**			
	44	29		15	0	0	29		15		100% (29/29)	100% (15/15)
TIB MOLBIOL VirSNiP SARS-CoV-2 Spike E484A assay	**Samples tested**	**Melting-curve analysis**					**WGS analysis**				**Sensitivity**	**Specificity**
		E484A		**E484E/K/Q**	**Ab Tm**	**No Tm**	**Omicron E484A**		**Others E484E/K/Q**			
	44	28		15	0	1*	29		15		96.6% (28/29)	100% (15/15)
TIB MOLBIOL VirSNiP SARS-CoV-2 Spike E484K assay	**Samples tested**	**Melting-curve analysis**					**WGS analysis**				**Sensitivity**	**Specificity**
		E484K	**E484Q**	**E484E**	**Ab Tm**	**No Tm**	**Beta E484K**	**Kappa E484Q**	**Omicron E484A**	**Others E484E**		
	41	4	3	12	22o1	0	4	3	22	12	100% (7/7)	100% (34/34)
TIB MOLBIOL VirSNiP SARS-CoV-2 Spike N501Y assay	**Samples tested**	**Melting-curve analysis**					**WGS analysis**				**Sensitivity**	**Specificity**
		N501Y		**N501N**	**Ab Tm**	**No Tm**	**Alpha, Beta, Omicron N501Y**			**Others**		
	41	8		10	6o2	17β,o3	31			10	25.8% (8/31)	100% (10/10)

Abbreviations: Ab Tm, number of samples with aberrant melting temperature; No Tm, number of samples dropped out with no melting peak nor temperature; WGS, whole genome sequencing.
β Beta variant with PHLSB in-house real-time RT-PCR Cp 34.19 (n = 1).
* Omicron variant with PHLSB in-house real-time RT-PCR Cp 37.56 (n = 1).
o1 Omicron variant (n = 22).
o2 Omicron variant (n = 6).
o3 Omicron variant (n = 16). Remark:β, * no amplification signal nor melting peak was observed, these were probably due to low SARS-CoV-2 viral concentration in the specimens.

Log_{10} dC/ml or below. Performances of the three evaluated sample-to-answer platforms were non-inferior to the conventional real-time RT-PCR assays in detecting SARS-CoV-2 Omicron variant (**Table 1**).

3.2. Test sensitivity and specificity of TIB MOLBIOL VirSNiP SARS-CoV-2 Spike S371L/S373P assay and TIB MOLBIOL VirSNiP SARS-CoV-2 Spike E484A assay

The spike S371L/S373P mutations of all 29 tested specimens with Omicron variant were detected by the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike S371L/S373P assay, with Cp values ranged from 21.66 to 40.78 in FAM channel and correct melting peaks at 62 ± 2°C (**Table 2**; sensitivity: 100%; n = 29/29). All non-Omicron SARS-CoV-2 specimens were detected as Spike S371S/S373S with correct melting peaks at 45°C (**Table 2**; specificity: 100%, n= 15/15). Representative melting peaks for the corresponding spike mutations were shown in **Fig. 1A**.

Among all tested specimens with Omicron variant, only twenty-eight specimens were detected with Spike E484A mutation by the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike E484A assay with correct melting peaks at >55°C (**Table 2**; sensitivity: 96.6%; n = 28/29; Tm ranged from 58°C to 60°C). All non-Omicron SARS-CoV-2 specimens were detected as Spike E484E/K/Q with correct melting peaks at 51°C, 46°C and 47.3°C respectively (**Table 2**; specificity: 100%; n = 15/15). Representative melting peaks for the corresponding spike mutations were shown in **Fig. 1B**.

3.3. Test sensitivity and specificity of TIB MOLBIOL VirSNiP SARS-CoV-2 Spike E484K assay and TIB MOLBIOL VirSNiP SARS-CoV-2 Spike N501Y assay

The spike E484K/Q mutations of all 7 tested specimens with Beta/Kappa variant were detected by the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike E484K assay, with correct melting peaks at 56-58°C

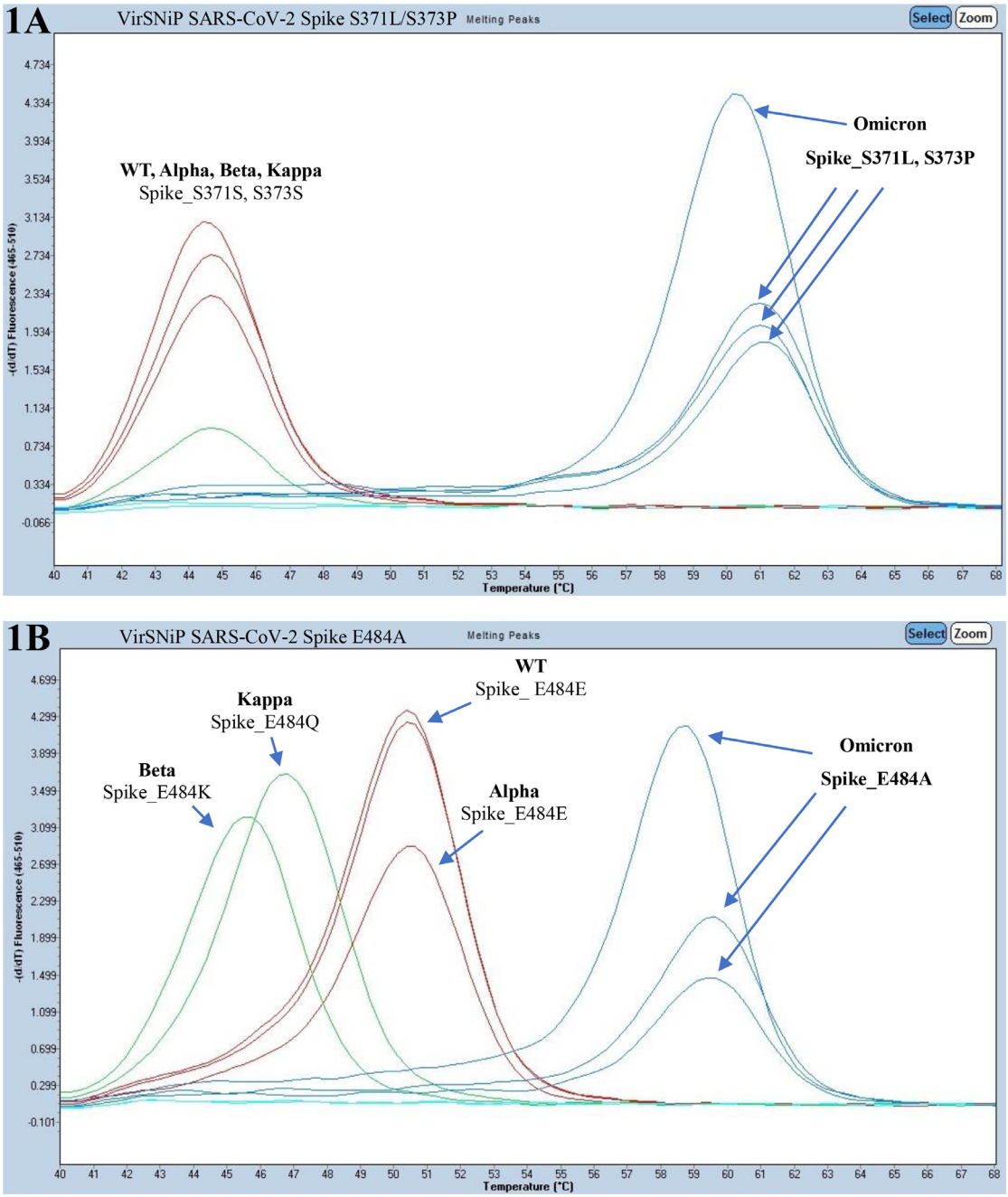

Fig. 1. Representative melting peaks of TIB MOLBIOL VirSNiP SARS-CoV-2 Spike S371L/S373P (Fig. 1A) and VirSNiP SARS-CoV-2 Spike E484A (Fig.1B) assays **(1A)** Correct melting peaks with Tm between 60-64°C were observed when Omicron specimens were tested with VirSNiP SARS-CoV-2 Spike S371L/S373P assay. **(1B)** Correct melting peaks with Tm >55°C were observed when Omicron specimens were tested with VirSNiP SARS-CoV-2 Spike E484A assay.

(**Table 2**; spike E484K-sensitivity: 100%; n = 4/4) or at 48-51°C (**Table 2**; spike E484Q-sensitivity: 100%; n = 3/3). Except Omicron specimens, all non-Beta/Kappa SARS-CoV-2 specimens were detected as Spike E484E with correct melting peaks at 52-55°C (**Table 2**; specificity: 100%, n= 34/34). Notably, an aberrant melting peak at Tm 42-44°C was observed when the specimens with Omicron variant were tested with the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike E484K assay (**Fig. 2A**).

The Spike N501Y mutations of 8 (out of 31) tested specimens with Alpha, Beta and Omicron variants were detected by the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike N501Y assay, with Cp values ranged from 21.86 to 38.85 in FAM channel and correct melting peaks at 60-62°C (**Table 2**; spike N501Y-sensitivity: 25.8%; n = 8/31). All non-Alpha/Beta/Omicron SARS-CoV-2 specimens were detected as Spike N501N with correct melting peaks at 55-57°C (specificity: 100%, n= 10/10). Notably, no amplification curve in FAM channel was observed in all twenty-two VOC-Omicron samples tested. Testing of six Omicron specimens gave an ambiguous melting peak with Tm within 54-55°C (**Fig. 2B**) whereas testing of the remaining sixteen VOC-Omicron specimens gave no melting peak.

4. Discussion

SARS-CoV-2 Omicron variant of concern (VOC) cases have been detected all over the world, and has recently displaced another VOC-Delta as the dominant variant in Africa [16]. The presence of mutations in the SARS-CoV-2 variant can potentially impact the performance of detec-

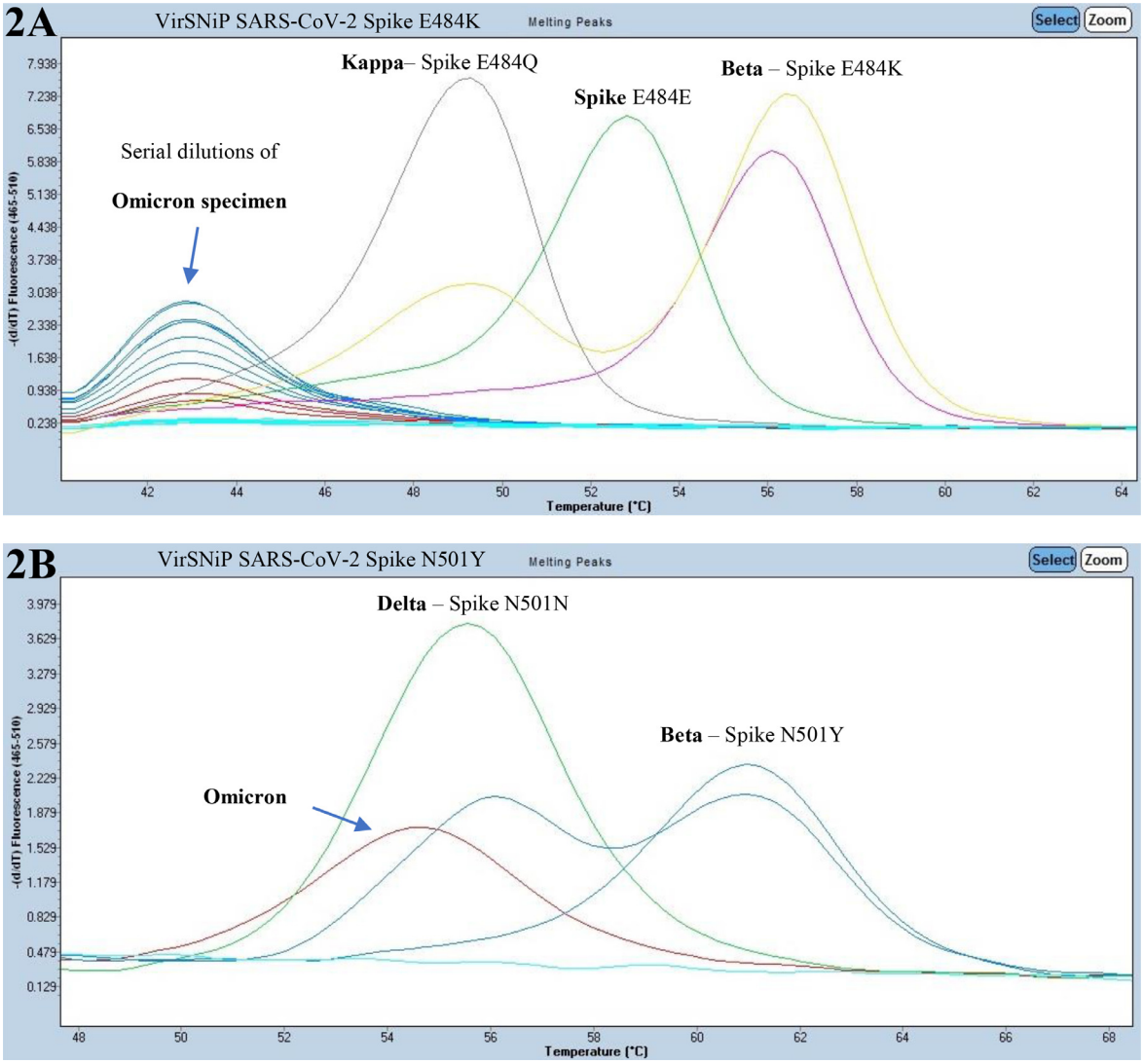

Fig. 2. Representative melting peaks of TIB MOLBIOL VirSNiP SARS-CoV-2 Spike Spike E484K (Fig. 2A) and VirSNiP SARS-CoV-2 Spike N501Y (Fig.2B) assays **(2A)** An aberrant melting peak with Tm between 42-44°C was observed when Omicron specimen was tested. The shift of melting temperature of VOC-Omicron may possibly be due to the presence of E484A mutation. **(2B)** An ambiguous melting peak indicated by blue arrow with Tm within 54-55°C.

tion test and false negative test results can occur as previously reported [7–9]. To our knowledge, our study is one of the very first to evaluate the SARS-CoV-2 VOC-Omicron detection performance of the commercial assays. In this study, the SARS-CoV-2 detection sensitivity of three commercial platforms, Roche cobas® 6800/8800, Roche cobas® Liat®, and Cepheid GeneXpert® systems were shown not to be impacted by the large number of mutations of VOC-Omicron BA.1.

There are limitations to this study that should be noted. First, only limited number of VOC-Omicron sample was included in this study. Second, newly emerged Omicron sub-lineages BA.2 and BA.3 were not available in Hong Kong during the study period and thus, were not included in this study. It is particularly worrying that two sub-lineages of this new variant emerged in such a short period of time in Africa, a rapid variant detection strategy combining rapid screening test and whole genome sequencing shall be encouraged to monitor and manage this alarming variant. In our study, we assessed four commercially available SARS-CoV-2 Spike SNP assays, which can be easily adapted in routine screening for Omicron variants. The signature mutations i.e. Spike S371L/Spike S373P and Spike E484A in VOC-Omicron BA.1 were correctly identified using the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike S371L/S373P and VirSNiP SARS-CoV-2 Spike E484A assays. Compared to whole genome sequencing, the evaluated TIB MOLBIOL VirSNiP SARS-CoV-2 Spike SNP assays are less labor-intensive and are of shorter test turnaround time (approximately 2.5 hours per test run), these allow high variant screening throughput and potentially facilitating rapid infection control response to avoid SARS-CoV-2 variant outbreak.

Notably, an aberrant melting peak with Tm between 42-44°C was observed when specimens with VOC-Omicron were tested with the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike E484K assay. Other notable phenomenon, including 1) presence of ambiguous melting peak at lower temperature or 2) absence of amplification curve was observed when the VOC-Omicron specimens were tested with the TIB MOLBIOL VirSNiP SARS-CoV-2 Spike N501Y assay. The spike E484 melting peak shift and unexpected spike N501Y dropout (probably caused by spike E484A mutation and mutations adjacent to spike N501 residue in Omicron variant), could potentially be used as markers for this variant, prior confirmation by whole genome sequencing.

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6. Ethics approval and consent to participate

Data records were de-identified and completely anonymous, so informed consent was waived.

7. Availability of data and materials

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcvp.2022.100091.

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