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Evaluation of the clinical and analytical performance of the Seegene allplex™ SARS-CoV-2 variants I assay for the detection of variants of concern (VOC) and variants of interests (VOI)

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ABSTRACT

Background: High-throughput assays for the detection of SARS-CoV-2 variants of concern (VOC) and interest (VOI) are a diagnostic alternative when whole genome sequencing (WGS) is unavailable or limited.
Objective: This study evaluated the clinical and analytical performance of the Seegene Allplex™ SARS-CoV-2 Variants I assay, which detects the HV69/70 deletion, N501Y and E484K mutations of the S gene.
Methods: Genotyping was evaluated on -871 SARS-CoV-2 RNA positive specimens, 408 nasopharyngeal (NP) swabs and 463 saline gargle (SG) specimens, with WGS used as the reference standard. Analytical performance was assessed including stability, reproducibility, limit of detection (LOD), cross-reactivity and interference with various respiratory microorganisms.
Results: The clinical study revealed sensitivity of 100% (95% CI 99.27%–100%) and specificity of 100% (95% CI 98.99%–100%) for HV69/70 deletion, sensitivity of 100% (95% CI 99.55%–100%) and specificity of 100% (95% CI 93.73% – 100%) for N501Y, and sensitivity of 100% (95% CI 98.94% – 100%) and specificity of 98.10% (95% CI 96.53% – 99.08%) for E484K mutation. The E484Q mutation was detected in 10 specimens of the Kappa variant (B.1.627.1). Analytical performance demonstrated stability and reproducibility over 7 days, and LOD was calculated at 698 cp/mL for NP swab specimens, and 968 cp/mL for SG specimens. No interference or cross-reactivity with other microorganisms was noted.
Conclusion: The Allplex™ SARS-CoV-2 Variants I assay is acceptable for clinical use for the detection of variant of concern and variant of interest.

1. Introduction

The coronavirus SARS-CoV-2 spread rapidly after its initial detection in Wuhan, China in December 2019, with a pandemic declared in March 2020. At the beginning of April 2020, a variant with the spike protein (S-protein) D614G mutation replaced the original SARS-CoV-2 strain in many areas of the world, as the mutation appears to improve the binding efficiency between the receptor binding domain (RBD) with the angiotensin-converting enzyme 2 (ACE2) receptor [1,2]. This mutation led to an enhanced replication and transmissibility in animal models of infection [2,3]. In September 2020, the B.1.1.7 lineage emerged as a variant of concern in the United Kingdom (UK), subsequently termed the alpha variant, with 9 spike protein mutations (del69/70HV, del144Y, N501Y, A570D, D614G, P681H, T761I, S982A, and D1118H) [4,5]. These mutations, specifically N501Y, A570D and D614G, appear to restructure the protein-protein interaction between the spike protein and the ACE2 receptor leading to an overall enhanced efficacy of cellular uptake [6–8]. Likewise, the B.1.1.7 variant has enhanced replication and transmissibility properties compared to the original Wuhan strain, which explained the rapid expansion in the UK and now in more than 114 countries [9–11]. Luckily, the B.1.1.7 variant remained sensitive to neutralizing antibodies and by serum samples from convalescent individuals and recipient of an mRNA vaccine, indicating that this variant is unlikely be a major concern for current vaccines efficacy [12,13]. Two

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subsequent variants of concern, B.1.351 (beta variant), first identified in South Africa, and P.1 (gamma variant), first identified in Brazil, were found to harbor the D614G and N501Y mutations, as well as 2 additional key mutations in the receptor binding domain (RBD), K417N/T and E484K, which increase binding affinity to the ACE2 receptor [14]. Similar to B.1.1.7, these strains appear to be more transmissible and infectious, and rapidly became predominant within their countries before global dissemination [11,15]. Furthermore, B.1.351 and P.1 have demonstrated immune escape from neutralizing antibodies from those with natural infection, as well as vaccine-induced immunity, which engender a treat to current vaccines efficacy [14,16–18]. Recently, variant delta (B.1.617.2), first identified in India, has rapidly spread in England and Scotland where it has outcompeted the variant alpha [19–22]. With ten different mutations in the S gene including L452R and D614G this variant showed an increased transmissibility and vaccine effectiveness is also reduced [21,23]. Other lineages, such as variant of interest (VOI) B.1.525 (Eta), B.1.526 (Iota), B.1.617.1 (Kappa) and C.37 (Lambda) also harbor mutations that are predicted to affect transmission, replication, and escape immunity [5,24]. A third class of SARS-CoV-2 variants named Alerts for Further Monitoring have been instated by WHO to capture any variants with genetic changes that are suspected to affect virus characteristics with some indication that it may pose a future risk [5]. This class includes B.1.427/B.1.429 and P.2 variants. Therefore, surveillance of VOC and VOI remains critical for adequate public health control measures and for investigation of vaccine effectiveness.

Seegene (Seoul, South Korea) has developed the Allplex™ SARS-CoV-2 Variants I real-time PCR assay for detection of mutations specific of VOCs from nasopharyngeal (NP) swabs, nasopharyngeal aspirate, bronchoalveolar lavage (BAL), sputum and oropharyngeal swabs [25,26]. The assay targets the RNA-dependent RNA polymerase (RdRp) gene, the S-protein N501Y and E484K mutations and HV69/70 deletion, as well as an endogenous internal control. In this study, we evaluated the analytical and clinical performance of the assay compared to whole genome sequencing (WGS) performed at a reference laboratory.

2. Materials and methods

2.1. Study site

Evaluation of the Seegene Allplex™ SARS-CoV-2 Variants I assay (Seegene, Seoul, South Korea) was performed at the Larissa Yarr Medical Microbiology Laboratory at Kelowna General Hospital, British Columbia (BC), Canada. WGS was performed at the British Columbia centre for Disease Control Public Health Laboratory (BCCDC PHL) in Vancouver, BC, Canada.

2.2. Assay procedures

The assays were performed according to manufacturer recommendations [26]. Specimens were heat inactivated at 65 °C for 20 min prior to nucleic acid extraction on a STARlet *in vitro* diagnostic (IVD) liquid handler, using Universal extraction kit and protocol (Seegene). Two hundred uL of specimens were used for nucleic acid extraction and eluated in 100 µL. Nucleic acid extracts were then set up for RT-PCR using the Allplex™ SARS-CoV-2 Variants I either manually or using the STARlet IVD Launcher OneStep program (Seegene) where 5 µL of extract were mixed with 5 µL of primers and probes, 5 µL of buffer and 5 µL of enzyme. Plates were run on CFX96™ IVD (BioRad, Hercules, USA) using the recommended protocol for the assay. Briefly, a reverse transcription step was initially performed at 50 °C for 20 min, then a denaturation step 95 °C for 15 min, initial cycling for 3 steps at 95 °C for 10 s, 60 °C for 40 s and 72 °C for 20 s were performed. Finally, 42 rapid cycles at 95 °C for 10 s, 60 °C for 15 s and 72 °C for 10 s were executed and fluorescence reads were recorded at 60 °C and 72 °C. Results interpretation and data analysis was achieved via the Seegene viewer.

Table 1
Summary of clinical specimens tested with the Allplex™ SARS-CoV-2 variants I assay and confirmed by WGS.

Classification§	Lineage	Mutations in S gene	NP swab (*n* = 408)	Saline gargle (*n* = 463)	Total
VOC	B.1.1.7 (Alpha)	Δ69/70, Δ144, (E484K*), (S494P*), N501Y, A570D, D614G, P681H, T716I, S982A, D1118H (K1191N*)	240	255	495
	P.1 (Gamma)	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I	126	176	302
	B.1.351 (Beta)	D80A, D215G, Δ241/242/243, K417N, E484K, N501Y, D614G, A701V	9	8	17
	B.1.627.2 (Delta)	T19R, (G142D*), 156del, 157del, R158G, L452R, T478K, D614G, P681R, D950N	0	2	2
VOI	B.1.627.1 (Kappa)	(T95I*), G145D, E154K, L452R, E484Q, D614G, P681R, Q1071H	5	5	10
	B.1.525 (Eta)	A67V, Δ69/70, Δ144, E484K, D614G, Q677H, F888L	6	5	11
	B.1.526 (Iota)	(L5F*), T95I, D253G, (S477N*), (E484K*), D614G, (A701V*)	3	1	4
Alerts for Further Monitoring	P.2	E484K, (F565L*), D614G, V1176F	2	1	3
	B.1.427	L452R, D614G	2	1	3
NON-VOC / NON-VOI	B.1.438.1	(E484K*), D614G, Q675H, C1254C	10	5	15
	B.1.2	Q677H	2	3	5
	Undetermined	N/A	3	1	4

VOC: Variant of concern; VOI: Variant of interest; NP: Nasopharyngeal swab.
* indicate the occasional presence of the mutation.
§ WHO classification of SARS-CoV-2 variants as of August 2021(19).

WGS was performed on a MiSeq or NextSeq instrument (Illumina, San Diego, USA) using the Freed 1200 bp amplicon scheme, as previously described [27].

2.3. Verification of clinical performance

Clinical performance of the RT-PCR assay was evaluated using specimens which tested positive for SARS-CoV-2 on the Seegene 2019-nCoV assay, Hologic Panther Fusion SARS-CoV-2 assay or GeneXpert SARS-CoV-2 assay between February 2021 and April 2021 from symptomatic patients. NP swabs in universal transport media (Yocon, Beijing, China) and saline gargle (SG) specimens were evaluated, with further details on SG collection published elsewhere [25]. WGS was performed on these specimens and sequence data generated were used as a

Table 2
Contingency table of clinical specimens tested for HV69/70 del, E484K, N501Y and RdRP gene compared to whole genome sequencing.

Target	WGS	Allplex™ SARS-CoV-2 Variants I assay		Sensitivity	Specificity
		Positive	Negative		
HV69/70 del	Positive	506	0	100% (95% CI 99.27% – 100%)	100% (95% CI 98.99% – 100%)
	Negative	0	365		
E484K	Positive	345	0	100% (95% CI 98.94% – 100%)	98.10% (95% CI 96.53% – 99.08%)
	Negative	10*	516		
N501Y	Positive	814	0	100% (95% CI 99.55% – 100%)	100% (95% CI 93.73% – 100%)
	Negative	0	57		
RdRP	Positive	871	0	100% (95% CI 99.58% – 100%)	N/A
	Negative	0	0		

* 10 B1.617 (E484Q) had a positive results when tested for E484K.

comparator. Sensitivity and specificity with their respective 95% confidence interval (95% CI) were calculated using contingency tables analysis and the two-sided Fisher's exact test from GraphPad Prism 6.01.

2.4. Validation of analytical performance

The analytical performance of the Allplex™ SARS-CoV-2 Variants I assay was assessed by evaluating the stability, reproducibility, limit of detection (LOD) and the cross-reactivity and interference with other microorganisms. Stability and reproducibility experiments were performed on NP swabs and SG specimens, by creating 1:100 dilutions with either a B.1.1.7-positive NP swab or a P.1-positive SG. Specimens were aliquoted in 500 µL tubes and left at 4 °C or 22 °C (room temperature) for 7 days. Aliquots were extracted and tested in duplicate at 0, 24, 48, 72, 144 and 168 h. The LOD was evaluated using synthetic DNA of the RdRP and the S gene (1–2160 nt) that harbored the HV69/70 del, E484K and N501Y (Genewiz, Seattle, USA). Simulated NP swab and SG specimens with known concentration of DNA (50, 100, 200, 300, 400, 500 and 1000 copies/mL) were extracted and tested at least 20 times for each concentration, with LOD calculated as previously described [28]. We determined the LOD of each specimen type as the concentration that was detected in at least 95% of the replicates ($LOD_{95\%}$). Cross-reactivity and interference were evaluated by testing 18 B1.1.7 and 18 P1 SARS-CoV-2 clinical specimens (NP and SG) spiked in a 1:3 ratio with specimens positive for other respiratory viruses, or oropharyngeal bacteria and fungi.

3. Results

3.1. Clinical performance

The Allplex™ SARS-CoV-2 Variants I assay was evaluated using 871clinical specimens, including 408 NP swabs and 463 saline gargle specimens. All specimens subsequently underwent WGS to confirm lineages and mutational profile (Table 1). The occasional mutation E484K reported in multiple lineages was only detected in the B.1.438.1 lineage where 8 out of 15 specimens had that specific mutation. No B.1.1.7 or B.1.526 strain harboured the E484K mutation in this data set. The analytical sensitivity of 100% (95% CI 99.27%−100%) and specificity of 100% (95% CI 98.99%−100%) for HV69/70 del, sensitivity of 100% (95% CI 99.55%−100%) and specificity of 100% (95% CI 93.73% – 100%) for N501Y, and sensitivity of 100% (95% CI 98.94% – 100%) and specificity of 98.10% (95% CI 96.53% – 99.08%) for E484K mutation (Table 2). The decreased specificity observed for the E484K target was due to the cross-reactivity with the E484Q mutation found in ten B.1.627.1 variants tested. The cycle threshold (Ct) values of the E484K positive target in the E484Q positive specimens increased by 6.15 cycles on average when compared to the Ct value for the RdRP gene (Table 3). In contrast, the E484K and RdRp Ct values were within 2 cycles for the P.1 and B.1.351 VOCs.

Table 3
Ct values of E484K and RdRP in 12 B1.617 specimens tested on the Allplex™ SARS-CoV-2 Variants I assay.

Sample	Lineage	sample type	E484K	RdRP gene	Δ CT (E484K-RdRP)	Average & St.D
1	B.1.617.1	NP	25.18	18.90	6.28	6.15 ± 0.48
2	B.1.617.1	NP	22.78	17.15	5.63	
3	B.1.617.1	NP	19.57	13.90	5.67	
4	B.1.617.1	SG	32.42	25.55	6.87	
5	B.1.617.2	SG	–	21.22	–	
6	B.1.617.1	SG	30.85	24.27	6.58	
7	B.1.617.2	SG	–	21.16	–	
8	B.1.617.1	NP	21.28	15.88	5.40	
9	B.1.617.1	SG	25.16	18.73	6.43	
10	B.1.617.1	NP	24.12	18.22	5.90	
11	B.1.617.1	SG	28.21	21.89	6.32	
12	B.1.617.1	SG	26.42	19.98	6.44	
13	P.1	NP	16.25	14.36	1.89	1.86 ± 0.03
14	P.1	SG	22.99	21.12	1.87	
15	P.1	SG	20.2	18.37	1.83	
16	P.1	NP	21.13	19.24	1.89	
17	P.1	NP	19.06	17.21	1.85	
18	P.1	NP	16.13	14.33	1.80	
19	P.1	NP	16.3	14.44	1.86	
20	B.1.351	NP	26.36	25.33	1.03	1.31 ± 0.27
21	B.1.351	SG	28.15	26.81	1.34	
22	B.1.351	SG	30.6	29.03	1.57	
23	B.1.525	NP	27.48	26.01	1.47	1.58 ± 0.26
24	B.1.525	SG	16.99	15.39	1.60	
25	B.1.525	SG	24.93	23.43	1.50	
26	B.1.525	NP	23.78	22.74	1.04	
27	B.1.525	SG	29.31	27.46	1.85	
28	B.1.525	SG	25.88	24.24	1.64	
29	B.1.525	NP	18.57	16.74	1.83	
30	B.1.525	SG	26.72	24.99	1.73	

Table 4
Analysis of Ct values of specimens stored under different conditions for 7 days.

Specimen	Analyte	Mean	%CV
NP at 4 °C for 7 days	E484K	23.59	1.85
	N501Y	23.76	1.69
	RdRP	22.13	1.37
NP at 22 °C for 7 days	E484K	23.60	1.75
	N501Y	23.60	1.87
	RdRP	22.13	1.34
SG at 4 °C for 7 days	HV69/70 del	24.23	1.62
	N501Y	26.88	1.50
	RdRP	25.44	1.60
SG at 22 °C for 7 days	HV69/70 del	24.33	1.99
	N501Y	26.81	1.32
	RdRP	25.53	1.74

NP: Nasopharyngeal swabs; SG: saline gargle.

3.2. Analytical performance

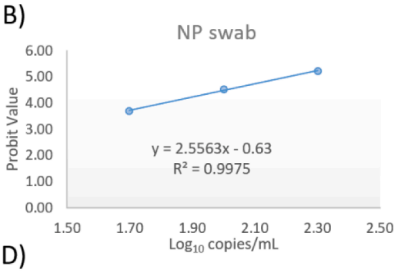
Stability studies demonstrated minimal variation when specimens were stored at either 4 °C or room temperature for 7 days (Table 4), reflecting high stability of both specimen types (NP swab and SG). This indicates that the Allplex™ SARS-CoV-2 Variants I assay can reproducibly detect targets on 7-day-old specimens with very little variation. LOD was calculated at 698 cp/mL for NP swabs and 968 cp/mL for SG specimens using synthetic DNA of the RdRP gene and the S gene (nt 1–2160) that harbor HV69/70 del, E484K and N501Y substitution (Fig. 1). Cross-reactivity and interference experiments revealed good performance as little variation in Ct values were observed in the presence of other potential respiratory pathogens (Table 5).

A)

NP

Copies/mL	Log10 copies/mL	No. of replicates	No. of positive	Positivity	Probit Value
1,000	3.0000	22	22	1.00	NA
500	2.6990	22	22	1.00	NA
200	2.3010	22	13	0.59	5.230
100	2.0000	22	7	0.32	4.527
50	1.6990	21	2	0.10	3.691

B)



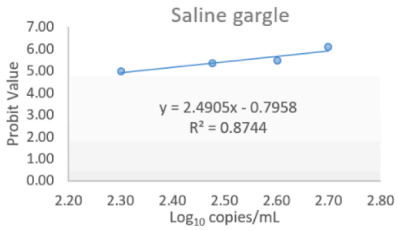
Slope	2.556
Intercept	-0.630
C95	2.844
LOD	698

C)

Saline Gargle

Copies/mL	Log10 copies/mL	No. of replicates	No. of positive	Positivity	Probit Value
1,000	3.0000	10	10	1.00	NA
500	2.6990	22	19	0.86	6.097
400	2.6021	22	15	0.68	5.473
300	2.4771	22	14	0.64	5.349
200	2.3010	10	5	0.50	5.000
100	2.0000	10	0	0.00	NA

D)



Slope	2.490
Intercept	-0.796
C95	2.986
LOD	968

Fig. 1. Limit of detection for A-B) nasopharyngeal (NP) swab and C-D) saline gargle specimen type. A) Positivity rate of SARS-CoV-2 variant targets detected in different concentration of synthetic DNA in NP specimen type. B) Probit analysis graph and calculation of LOD for NP specimen type. C) Positivity rate of SARS-CoV-2 variant targets detected in different concentration of synthetic DNA in saline gargle specimen type. D) Probit analysis graph and calculation of LOD for saline gargle specimen type.

Table 5
Analysis of Ct values obtained with and without the presence of interferent microorganisms.

Specimen	Lineage	Interferent	E484K			RdRP			N501Y			HV69/70 del		
			Mean	St.D	% CV	Mean	St.D	% CV	Mean	St.D	% CV	Mean	St.D	% CV
NP	P.1	*Candida albicans*	17.68	0.16	0.88	16.15	0.01	0.09	18.01	0.13	0.71			
NP	P.1	*Pseudomonas aeruginosa*	19.89	0.99	4.98	18.29	0.59	3.25	19.67	0.34	1.73			
NP	P.1	Influenza A virus type H3	19.24	0.10	0.51	17.92	0.03	0.16	19.58	0.11	0.58			
NP	P.1	Influenza A virus type H1N1-2009	22.40	0.01	0.03	21.00	0.10	0.47	22.67	0.01	0.03			
NP	P.1	Influenza B virus	18.70	0.11	0.61	17.29	0.23	1.35	18.96	0.20	1.04			
NP	P.1	Adenovirus	15.73	0.11	0.72	14.32	0.18	1.23	16.00	0.14	0.88			
NP	P.1	*Chlamydophila pneumoniae*	15.89	0.08	0.53	14.47	0.06	0.39	16.07	0.07	0.44			
NP	P.1	Parainfluenza virus type 1 & 2 and Rhinovirus	14.42	0.43	2.99	12.65	0.20	1.57	14.86	0.45	3.05			
NP	P.1	Bocavirus and Parainfluenza virus type 4	14.43	0.21	1.47	13.23	0.06	0.48	14.85	0.21	1.43			
NP	B.1.1.7	Metapneumovirus and Rhinovirus				12.38	0.15	1.20	14.37	0.17	1.18	10.90	0.62	5.65
NP	B.1.1.7	Parainfluenza virus type 2				14.21	0.20	1.39	15.79	0.35	2.20	12.71	0.17	1.34
NP	B.1.1.7	Parainfluenza virus type 1 & 2				19.58	0.06	0.33	21.14	0.11	0.50	18.28	0.37	2.01
NP	B.1.1.7	Rhinovirus/Enterovirus				15.50	0.10	0.64	17.24	0.18	1.07	14.64	0.06	0.39
NP	B.1.1.7	Coronavirus NL63				21.20	0.18	0.83	22.75	0.45	1.99	20.11	0.35	1.72
NP	B.1.1.7	Coronavirus HKU1				17.86	0.16	0.87	19.59	0.33	1.70	16.60	0.04	0.26
NP	B.1.1.7	Metapneumovirus				14.44	0.12	0.83	15.95	0.11	0.71	12.62	0.04	0.34
NP	B.1.1.7	Rhinovirus and Coronavirus HKU1				12.52	0.06	0.51	14.72	0.08	0.58	10.96	0.05	0.45
NP	B.1.1.7	Respiratory Syncytial Virus type A, Bocavirus and Rhinovirus				16.86	0.08	0.50	18.51	0.04	0.19	15.89	0.13	0.80
SG	P.1	*Candida albicans*	21.12	0.24	1.14	19.23	0.15	0.77	21.10	0.08	0.37			
SG	P.1	*Pseudomonas aeruginosa*	22.79	1.08	4.75	20.96	0.87	4.15	22.72	0.54	2.37			
SG	P.1	Influenza A virus type H3	24.62	0.30	1.21	23.11	0.09	0.40	24.52	0.17	0.69			
SG	P.1	Influenza A virus type H1N1-*2009	25.33	0.18	0.70	23.38	0.13	0.54	24.86	0.10	0.40			
SG	P.1	Influenza B virus	23.57	0.21	0.87	21.92	0.10	0.45	23.24	0.18	0.76			
SG	P.1	Rhinovirus/Enterovirus	24.28	0.50	2.07	22.86	0.01	0.06	24.32	0.09	0.38			
SG	P.1	Coronavirus NL63	27.81	0.04	0.13	26.54	0.04	0.13	27.82	0.01	0.05			
SG	P.1	Coronavirus HKU1	25.10	0.03	0.11	23.28	0.19	0.82	24.57	0.06	0.23			
SG	P.1	Rhinovirus and Coronavirus HKU1	32.20	0.52	1.60	30.54	0.04	0.12	32.11	0.65	2.03			
SG	B.1.1.7	Adenovirus				13.87	0.80	5.76	15.62	0.92	5.89	12.15	0.95	7.86
SG	B.1.1.7	Rhinovirus				23.07	0.19	0.83	24.21	0.14	0.58	22.85	0.21	0.90
SG	B.1.1.7	Metapneumovirus and Rhinovirus				19.82	0.18	0.89	21.62	0.20	0.92	19.75	0.21	1.07
SG	B.1.1.7	*Chlamydophila pneumoniae*				28.43	0.35	1.24	29.50	0.13	0.46	28.34	0.18	0.65
SG	B.1.1.7	Parainfluenza virus type 2				23.53	0.06	0.24	24.85	0.05	0.20	23.13	0.22	0.95
SG	B.1.1.7	Parainfluenza virus type 1 & 2				22.33	0.09	0.41	23.60	0.16	0.69	21.67	0.13	0.62
SG	B.1.1.7	Parainfluenza virus type 1 & 2 and Rhinovirus				16.66	0.08	0.51	18.28	0.05	0.27	15.94	0.06	0.40
SG	B.1.1.7	Bocavirus and Parainfluenza virus type 4				22.22	0.08	0.38	23.46	0.08	0.33	21.46	0.00	0.00
SG	B.1.1.7	Respiratory Syncytial Virus type A, Bocavirus and Rhinovirus				19.65	0.11	0.58	21.46	0.16	0.72	19.03	0.00	0.00

NP: Nasopharyngeal swabs; SG: saline gargle; St.D: Standard deviation;% CV: coefficient of variation in percentage.

4. Discussion

This study that included 871 clinical SARS-CoV-2 specimens, demonstrated the high clinical and analytical performance of the Seegene Allplex™ SARS-CoV-2 Variants I assay for the detection of SARS-CoV-2 viruses containing HV69/70 deletion, E484K and N501Y mutations in the S gene, demonstrating its suitability for clinical testing. This adds to existing literature, with only one other recent study reporting use of the Allplex™ SARS-CoV-2 Variants I assay on 30 nasopharyngeal swab specimens, where 4 variants belonging to R.1 lineage were detected [29]. The SARS-CoV-2 mutations targeted by the Allplex assay are currently found in VOC lineages B.1.1.7, P1, B.1.351 and VOI lineages P.2, P.3, B.1.525, B.1.526 and R.1. However, this new assay cannot detect mutations found in B.1.627.2, B.1.627.1, B.1.427/B.1.429 and C.37. Although distinction between lineages that harbor similar mutations can only be achieved by WGS, our results demonstrated that close to 99% of all specimens tested on the Allplex™ SARS-CoV-2 Variants I assay were corroborated by WGS. Interestingly, the 10 specimens discrepant for the E484K target were B.1.627.1 VOCs, which harbor the E484Q mutation. The detection of E484Q mutation was delayed by 6.15 Ct values on average when compared to the RdRP gene, facilitating differentiation from true positive E484K mutations in most cases, in which the Ct value was within 2 cycles. However, these results were generated from a limited number of samples and a cross reaction of E484Q with E484K primers and probes has been previously reported [30]. The assay also performed well in analytical examination, demonstrating stability and reproducibility of results over 7 days under various storage conditions, and an acceptable LOD and no cross-reactivity or interference by other respiratory viruses or oropharyngeal bacteria and fungi.

Detection of specific S gene mutations of concern within SARS-CoV-2 by high throughput RT-PCR assays is an effective approach to capture circulating VOC and VOI when whole genome sequencing is unavailable or limited. Considering the increased cost, and specialized human resources and infrastructure demands associated with WGS, targeted PCR for the detection of several VOC/VOI is a practical option. This approach provides a rapid turnaround time (~ 5 h from nucleic acid extraction to result) and leverages testing capacity that is available in clinical laboratories. Other commercial tests are available for the detection of variants of concern or variant of interest. Seegene has also launched the Allplex™ SARS-CoV-2 Master assay that can detect the presence of SARS-CoV-2 via the detection of E, N and RdRP genes and the presence of S gene mutations such as HV69/70 deletion, Y144 deletion, E484K, N501Y and P681H. Given that all the S gene mutations/deletions are detected in the same channel (Quasar 705), it is impossible to distinguish a probable B.1.1.7 specimen from a probable P.1 or B.1.351, limiting its utility [31]. ThermoFisher Scientific has released the TaqMan SARS-CoV-2 Mutation Panel where you can choose from 26 duplex assays for detection of various mutations [32]. BGI also offer various kit that can detect signature mutations found in B.1.1.7, B.1.351 and P.1 lineages [33]. These assays have been designed for research use only and no clinical and analytical performance were available at the time of the drafting of this manuscript. Considering the dynamic nature of SARS-CoV-2, the ability to add or modify new targets is critical for the suitability of VOC/VOI detection by single nucleotide polymorphism PCR. Laboratory developed tests often offers this flexibility. For example, laboratory developed tests for the detection of L484R, E484K and N501Y (alone or in multiplex) in nasopharyngeal swabs specimen were reported earlier this year, demonstrating excellent clinical performance, and provide an even more economical option in laboratories that have the expertise and infrastructure to validate and perform them [34–37]. Given surveillance of circulating VOC/VOI remains critical for adequate public health control measures and for investigation of vaccine effectiveness when WGS is unavailable or limited, the Seegene Allplex™ SARS-CoV-2 Variants I assay offers a suitable alternative.

Declaration of competing interest

All 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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References

[1] B. Korber, W.M. Fischer, S. Gnanakaran, H. Yoon, J. Theiler, W. Abfalterer, et al., Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus, Cell [Internet] 182 (4) (2020) 812–827. Aug 20 [cited 2021 Jun 9]e19. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7332439/.

[2] B. Zhou, T.T.N. Thao, D. Hoffmann, A. Taddeo, N. Ebert, F. Labroussaa, et al., SARS-CoV-2 spike D614G change enhances replication and transmission, Nature [Internet] 592 (7852) (2021) 122–127. Apr [cited 2021 Jun 9]Available from: https://www.nature.com/articles/s41586-021-03361-1.

[3] J.A. Plante, Y. Liu, J. Liu, H. Xia, B.A. Johnson, K.G. Lokugamage, et al., Spike mutation D614G alters SARS-CoV-2 fitness, Nature [Internet] 592 (7852) (2021) 116–121. Apr [cited 2021 Jun 9]Available from: https://www.nature.com/articles/s41586-020-2895-3.

[4] S.E. Galloway, P. Paul, D.R. MacCannell, M.A. Johansson, J.T. Brooks, A. MacNeil, et al., Emergence of SARS-CoV-2 B1.1.7 lineage — United States, December 29, 2020–January 12, 2021, Morb. Mortal Wkly. Rep. [Internet] 70 (3) (2021) 95–99. Jan 22 [cited 2021 Jun 9]Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7821772/.

[5] Tracking SARS-CoV-2 variants [Internet]. [cited 2021 Aug 9]. Available from: https://www.who.int/emergencies/emergency-health-kits/trauma-emergency-surgery-kit-who-tesk-2019/tracking-SARS-CoV-2-variants2021.

[6] E. Socher, M. Conrad, L. Heger, F. Paulsen, H. Sticht, F. Zunke, et al., Mutations in the B.1.1.7 SARS-CoV-2 spike protein reduce receptor-binding affinity and induce a flexible link to the fusion peptide, Biomedicines [Internet] 9 (5) (2021). May 8 [cited 2021 Jun 9]Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8151884/.

[7] D.A. Ostrov, Structural consequences of variation in SARS-CoV-2 B1.1.7, J. Cell Immunol. [Internet] 3 (2) (2021) 103–108 [cited 2021 Jun 9]Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8104447/.

[8] B. Luan, H. Wang, T. Huynh, Enhanced binding of the N501Y-mutated SARS-CoV-2 spike protein to the human ACE2 receptor: insights from molecular dynamics simulations, FEBS Lett. [Internet] 595 (10) (2021) 1454–1461. May [cited 2021 Jun 10]Available from: https://onlinelibrary.wiley.com/doi/10.1002/1873-3468.14076.

[9] E. Volz, S. Mishra, M. Chand, J.C. Barrett, R. Johnson, L. Geidelberg, et al., Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England, Nature [Internet] 593 (7858) (2021) 266–269. May [cited 2021 Jun 9]Available from: https://www.nature.com/articles/s41586-021-03470-x.

[10] N.G. Davies, S. Abbott, R.C. Barnard, C.I. Jarvis, A.J. Kucharski, J.D. Munday, et al., Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England, Science [Internet] 372 (6538) (2021). Apr 9 [cited 2021 Jun 9]Available from: https://science.sciencemag.org/content/372/6538/eabg3055.

[11] Á. O'Toole, V. Hill, O.G. Pybus, A. Watts, I.I. Bogoch, K. Khan, et al., Tracking the international spread of SARS-CoV-2 lineages B.1.1.7 and B.1.351/501Y-V2, Wellcome Open Res. [Internet] 6 (2021). May 19 [cited 2021 Jun 9]Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8176267/.

[12] X. Shen, H. Tang, C. McDanal, K. Wagh, W. Fischer, J. Theiler, et al., SARS-CoV-2 variant B.1.1.7 is susceptible to neutralizing antibodies elicited by ancestral spike vaccines, Cell Host Microbe [Internet] 29 (4) (2021) 529–539. Apr 14 [cited 2021 Jun 9]e3. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7934674/.

[13] D. Planas, T. Bruel, L. Grzelak, F. Guivel-Benhassine, I. Staropoli, F. Porrot, et al., Sensitivity of infectious SARS-CoV-2 B1.1.7 and B.1.351 variants to neutralizing antibodies, Nat. Med. [Internet] 27 (5) (2021) 917–924. May [cited 2021 Jun 9] Available from: https://www.nature.com/articles/s41591-021-01318-5.

[14] A. Khan, T. Zia, M. Suleman, T. Khan, S.S. Ali, A.A. Abbasi, et al., Higher infectivity of the SARS-CoV-2 new variants is associated with K417N/T, E484K, and N501Y mutants: an insight from structural data, J. Cell Physiol. [Internet] (2021) [citedJun 9];n/a(n/a). Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/jcp.30367.

[15] N.R. Faria, T.A. Mellan, C. Whittaker, I.M. Claro, S. Candido D da, S. Mishra, et al., Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil, Science [Internet] 372 (6544) (2021) 815–821. May 21 [cited 2021 Jun 9] Available from: https://science.sciencemag.org/content/372/6544/815.

[16] S.S. Abdool Karim, T de Oliveira, New SARS-CoV-2 variants — clinical, public health, and vaccine implications, N. Engl. J. Med. [Internet] 384 (19) (2021) 1866–1868. May 13 [cited 2021 May 21]Available from: http://www.nejm.org/doi/10.1056/NEJMc2100362.
[17] M. Hoffmann, P. Arora, R. Groß, A. Seidel, B.F. Hörnich, A.S. Hahn, et al., SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies, Cell [Internet]. 184 (9) (2021) 2384–2393. Apr 29 [cited 2021 Jun 9]e12. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7980144/.
[18] D. Zhou, W. Dejnirattisai, P. Supasa, C. Liu, A.J. Mentzer, H.M. Ginn, et al., Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera, Cell [Internet] 184 (9) (2021) 2348–2361. Apr 29 [cited 2021 Jun 9] e6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7901269/.
[19] Tracking SARS-CoV-2 variants [Internet]. [cited 2021 Jun 18]. Available from: https://www.who.int/activities/tracking-SARS-CoV-2-variants2021.
[20] H. Allen, A. Vusirikala, H. Flannagan, K.A. Twohig, A. Zaidi, N. Groves, et al., Increased Household Transmission of COVID-19 Cases associated with SARS-CoV-2 variant of concern B.1.617.2: a national case-control study, Public Health England (2021).
[21] A. Sheikh, J. McMenamin, B. Taylor, C Robertson, SARS-CoV-2 Delta VOC in Scotland: demographics, risk of hospital admission, and vaccine effectiveness, Lancet [Internet] (2021). Jun [cited 2021 Jun 18]; Available from, https://linkinghub.elsevier.com/retrieve/pii/S0140673621013581.
[22] I. Torjesen, Covid-19: delta variant is now UK's most dominant strain and spreading through schools, BMJ [Internet] (2021). Jun 4 [cited 2021 Jun 18]; n1445. Available from: https://www.bmj.com/lookup/doi/10.1136/bmj.n1445.
[23] V.V. Edara, B.A. Pinsky, M.S. Suthar, L. Lai, M.E. Davis-Gardner, K. Floyd, et al., Infection and vaccine-induced neutralizing-antibody responses to the SARS-CoV-2 B1.617 variants, N. Engl. J. Med. [Internet] (2021). Jul 7 [cited 2021 Aug 9]; Available from, https://pubmed.ncbi.nlm.nih.gov/34233096/.
[24] CDC. Coronavirus disease 2019 (COVID-19) [Internet]. centers for disease control and prevention. 2020 [cited 2021 Jun 4]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html.
[25] D.M. Goldfarb, P. Tilley, G.N. Al-Rawahi, J.A. Srigley, G. Ford, H. Pedersen, et al., Self-collected saline gargle samples as an alternative to health care worker-collected nasopharyngeal swabs for COVID-19 diagnosis in outpatients, J. Clin. Microbiol 59 (4) (2021) 7.
[26] Seegene. NovaplexTM SARS-CoV-2 variants I assay (RUO). 2021.
[27] Hogan C.A., Sbihi H., Jassem A., Tyson J.R., Lee T.D., Tsang F., et al. Optimizing SARS-CoV-2 variant of concern screening: experience from British Columbia, Canada, early 2021.:18.
[28] E.M. Burd, Validation of laboratory-developed molecular assays for infectious diseases, Clin. Microbiol. Rev. [Internet] 23 (3) (2010) 550–576. Jul [cited 2021 Jun 9]Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2901657/.
[29] W. Kami, T. Kinjo, W. Arakaki, H. Oki, D. Motooka, S. Nakamura, et al., Rapid and simultaneous identification of three mutations by the NovaplexTM SARS-CoV-2 variants I assay kit, J. Clin. Virol. [Internet] (2021). May [cited 2021 Jun 10]; 104877. Available from: https://linkinghub.elsevier.com/retrieve/pii/S138665322100144X.
[30] M. Verghese, B. Jiang, N. Iwai, M. Mar, M.K. Sahoo, F. Yamamoto, A SARS-CoV-2, Variant with L452R and E484Q neutralization resistance mutations [Internet], J. Clin. Microbiol. (2021) [citedAug 18]. Available from: https://journals.asm.org/doi/abs/10.1128/JCM.00741-21.
[31] Seegene Inc [Internet]. [cited 2021 Jun 10]. Available from: https://www.seegene.com/assays/allplex_sars-cov-2_master_assay2021.
[32] TaqMan SARS-CoV-2 Mutation Panel - CA [Internet]. [cited 2021 Jun 9]. Available from: https://thermofisher.com/ca/en/home/clinical/clinical-genomics/pathogen-detection-solutions/real-time-pcr-research-solutions-sars-cov-2/mutation-panel.html2021.
[33] SARS-CoV-2 Variant Identification Panel [Internet]. BGI - US. [cited 2021 Jun 10]. Available from: https://www.bgi.com/us/sars-cov-2-variant-detection/2021.
[34] Y. Zhao, A. Lee, K. Composto, M.H. Cunningham, J.R. Mediavilla, S. Fennessey, et al., A novel diagnostic test to screen SARS-CoV-2 variants containing E484K and N501Y mutations, Emerg. Microbes Infect. [Internet] 10 (1) (2021) 994–997 [citedJun 9]Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8168736/.
[35] M. Sandoval Torrientes, C. Castelló Abietar, J. Boga Riveiro, M.E. Álvarez-Argüelles, S. Rojo-Alba, F. Abreu Salinas, et al., A novel single nucleotide polymorphism assay for the detection of N501Y SARS-CoV-2 variants, J. Virol. Methods [Internet] (2021). Mar 24 [cited 2021 Jun 10]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7989071/.
[36] H. Wang, J.A. Miller, M. Verghese, M. Sibai, D. Solis, K.O. Mfuh, et al., Multiplex SARS-CoV-2 genotyping RT-PCR for population-level variant screening and epidemiologic surveillance, J. Clin. Microbiol. [Internet] (2021). May 26 [cited 2021 Jun 10]; Available from: https://journals.asm.org/doi/10.1128/JCM.00859-21.
[37] G. Cabecinhas, A. Rita, T. Roloff, M. Stange, C. Bertelli, M. Huber, et al., SARS-CoV-2 N501Y introductions and transmissions in Switzerland from beginning of October 2020 to February 2021—implementation of swiss-wide diagnostic screening and whole genome sequencing, Microorganisms [Internet] 9 (4) (2021) 677. Apr [cited 2021 Jun 11]Available from: https://www.mdpi.com/2076-2607/9/4/677.