Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
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)

Mélissa Caza¹,*, Catherine A. Hogan⁵, Agatha Jassem⁵, Natalie Prystajecky⁵, Amir Hadzic¹, Amanda Wilmer¹

¹ Larissa Yarr Medical Microbiology Laboratory, Kelowna General Hospital, Kelowna, British Columbia, Canada
² British Columbia Centre for Disease Control Public Health Laboratory, Vancouver, British Columbia, Canada
³ Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada

ARTICLE INFO
Keywords:
SARS-CoV-2  
COVID-19  
Variants of concern (VOC)  
Variants of interest (VOI)  
RT-PCR  
diagnostic  

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].

* Corresponding author at: Larissa Yarr Medical Microbiology Laboratory, Kelowna General Hospital, 2268 Pandosy Street, Kelowna, BC, V1Y 1T2.
E-mail address: Melissa.Caza@interiorhealth.ca (M. Caza).

https://doi.org/10.1016/j.jcv.2021.104996
Received 21 June 2021; Received in revised form 21 August 2021; Available online 2 October 2021
1386-6532/© 2021 Elsevier B.V. All rights reserved.
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 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 Kellowna 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 μl. of specimens were used for nucleic acid extraction and eluted 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 transcrip-tion 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.

| Classification | Lineage | Mutations in S gene | NP swab | Saline gargle | VOC: Variant of concern; VOI: Variant of interest; NP: Nasopharyngeal swab. |
|----------------|---------|---------------------|---------|--------------|---------------------------------------------------------------------|
|              | B.1.1.7 | Δ69/70, Δ144, (E484K), (E484K*), N501Y, A570D, D614G, P681H, T176I, S982A, D1181H (K191N*) | 240     | 255          | 495                                                                |
| VOC: Variant of concern | B.1.351 (Iota) | Δ69/70, Δ144, (E484K), (E484K*), N501Y, A570D, D614G, P681H, T176I, S982A, D1181H (K191N*) | 9       | 8            | 17                                                                 |
|              | B.1.526.1 (Kappa) | Δ69/70, Δ144, (E484K), (E484K*), N501Y, A570D, D614G, P681H, T176I, S982A, D1181H (K191N*) | 6       | 5            | 11                                                                 |
|              | B.1.526 (Iota) | Δ69/70, Δ144, (E484K), (E484K*), N501Y, A570D, D614G, P681H, T176I, S982A, D1181H (K191N*) | 3       | 1            | 4                                                                  |
|              | B.1.427 | Δ69/70, Δ144, (E484K), (E484K*), N501Y, A570D, D614G, P681H, T176I, S982A, D1181H (K191N*) | 2       | 1            | 3                                                                  |
|              | B.1.438.1 | Δ69/70, Δ144, (E484K), (E484K*), N501Y, A570D, D614G, P681H, T176I, S982A, D1181H (K191N*) | 10      | 5            | 15                                                                 |
|              | B.1.2 | Δ69/70, Δ144, (E484K), (E484K*), N501Y, A570D, D614G, P681H, T176I, S982A, D1181H (K191N*) | 2       | 3            | 5                                                                  |
|              | Undetermined | Δ69/70, Δ144, (E484K), (E484K*), N501Y, A570D, D614G, P681H, T176I, S982A, D1181H (K191N*) | 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 (Yoon, 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
**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 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].

Stability studies demonstrated minimal variation when specimens 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 substitution (Table 1). The occasional mutation E484K and RdRp Ct values were within 2 cycles for the 7-day-old specimens with very little variation. This indicates that the Allplex™ SARS-CoV-2 Variants I assay can reproducibly detect targets on 7-day-old specimens with very little variation. 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).

**3. Results**

**3.1. Clinical performance**

The Allplex™ SARS-CoV-2 Variants I assay was evaluated using 871 clinical 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 and RdRp Ct values were within 2 cycles for the 7-day-old specimens with very little variation. This indicates that the Allplex™ SARS-CoV-2 Variants I assay can reproducibly detect targets on 7-day-old specimens with very little variation.

| Target       | WGS   | Allplex™ SARS-CoV-2 Variants I assay | Sensitivity | Specificity |
|--------------|-------|-------------------------------------|-------------|-------------|
| HV69/70      | Positive | 100% (95% CI 98.99% – 100%) | 100%         |
|              | Negative | 99.5% – 100% | 100%         |
| E484K        | Positive | 100% (95% CI 98.10% – 100%) | 100%         |
|              | Negative | 98.94% – CI 96.53% | 99.08%       |
| N501Y        | Positive | 100% (95% CI 100% – 100%) | 100%         |
|              | Negative | 99.5% – 93.73% | 100%         |
| RdRP         | Positive | 100% (95% CI 100% – 100%) | 100%         |
|              | Negative | 99.58% – N/A | 100%         |

Table 2

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

| 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 | 1.52 |
|           | RdRp   | 25.53 | 1.74 |

Table 3

Ct values of E484K and RdRp in 12 B.1.617 specimens tested on the Allplex™ SARS-CoV-2 Variants I assay.

**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).
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                      | E4B4K | St.D | % CV | RdRP | St.D | % CV | N501Y | St.D | % CV | HV69/70 del | 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 | 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 pneumonia         | 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 |              |      |      |      |
| 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 | Parainfluenza virus type 1 & 2  | 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 | Influenza A virus type H1N1-2009| 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, P.1, 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 discordant 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. Considering the increased cost, and specialized human resources required, high throughput RT-PCR assays is an effective approach to capture circulating VOC and VOI when whole genome sequencing is unavailable. Limiting its utility [31]. Thermo Fisher Scientific has released the TaqMan SARS-CoV-2 Mutation Panel which can be used to detect from 26 duplex assays for detection of variants [32] with 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 required, high throughput RT-PCR assays is an effective approach to capture circulating VOC and VOI when whole genome sequencing is unavailable. Limiting its utility [31]. ThermoFisher Scientific has released the TaqMan SARS-CoV-2 Mutation Panel which can be used to detect from 26 duplex assays for detection of various mutations [32], BGI also offers various kits 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 mutations (alone or in multiplex) in nasopharyngeal swabs specimens 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.

Acknowledgments

We thank the KGH laboratory molecular technologists for performing PCR testing on clinical specimens. We thank the BCCDC PHL technologists from the Bacteriology and Advanced Mycology, and Virology laboratories, for validation panel preparation and WGS testing, and the informatics team for WGS analysis. We also thank Yin Chang for her assistance in organizing and providing WGS data.

References

[1] B. Korber, W.M. Fischer, S. Gnanakaran, H. Yoon, J. Theiler, W. Afsharifar, 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]Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7582439/.
[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/s41586-021-03185-x.
[3] J.A. Plante, Y. Liu, J. Liu, X. 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/article/s41586-020-0985-z.
[4] S.E. Galloway, P. Paul, D.R. MacCannell, M.A. Johansson, J.T. Brooks, A. MacNeil, et al., Emergence of SARS-CoV-2 B.1.1.7 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-tek-2019-tracking-SARS-CoV-2-variations/.
[6] E. Socher, M. Conrad, L. Heger, F. Paulsen, H. Sticht, F. Junke, 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 B.1.1.7, J. Cell. Immunol. [Internet] 3 (2) (2021) 103–108 [cited 2021 Jun 9]Available from: http://dx.doi.org/10.1098/rscb.2021.0006.
[8] B. Luan, H. Wang, T. Huyhn, 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) 1456–1461. May [cited 2021 Jun 10]Available from: https://onlinelibrary.wiley.com/doi/10.1002/1477-282X.
[9] E. Voiz, S. Mishra, M. Chand, J.C. Barrett, R. Johnson, L. Geidelberg, et al., Assessing transmissibility and impact of SARS-CoV-2 lineages B.1.1.7 in England, Nature [Internet] 593 (7868) (2021) 266–269. May [cited 2021 Jun 9]Available from: https://www.nature.com/articles/s41586-021-03361-1.
[10] M. Caza et al., Tracking SARS-CoV-2 variants in Canada, PLoS One [Internet] 16 (5) (2021). May 21 [cited 2021 Jun 9]Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8316419/.
[11] G.J. Hart, T. Gold, T.R. Stothard, M. Depledge, P. Barua, A. Watts, et al., Tracking 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/.
[12] A. Winchester, P. Smith, A. Williams, S. Greenhalgh, S. Karmali, et al., Tracking 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/.
[13] D. Planas, T. Brud, L. Grzelak, F. Guivel-Benhassine, I. Stapporn, F. Porrot, et al., Sensitivity of infectious SARS-CoV-2 B1.1.7 and B.1.351 variants to neutralizing antibodies, Cell [Internet] 182 (4) (2020) 812–827. Aug 20 [cited 2021 Jun 9]Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7733769/.
[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 mutations: an insight from structural data, J. Cell Physiol. [Internet] 269. May [cited 2021 Jun 9]Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8433081/.
[15] N.R. Faria, T.A. Mellan, C. Whittaker, L.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.

5
M. Caza et al.

[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/NEJMct2100362.

[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] Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7901444/.

[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) 2348–2351. Apr 29 [cited 2021 Jun 9] 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-variants

[20] H. Allen, A. Vasirikala, 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 Canada, early 2021.:18. Available from: https://www.bmj.com/lookup/doi/10.1136/bmj.n1445.

[22] 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/.

[23] 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/S138665392100144X.

[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] E. M. Pinto, M. A. Pinto, S. Pinto, C. Pinto, J. Pinto, A. Pinto, et al., Evidence of escape of SARS-CoV-2, Variant with L452R and E484Q neutralization resistance mutations [Internet], J. Clin. Microbiol. (2021) [cited Aug 18]. Available from: https://journals.asm.org/doi/abs/10.1128/JCM.00741-21.

[26] Seegene Inc [Internet]. [cited 2021 Jun 10]. Available from: https://www.seegene.com/assays/allplex_sars-cov-2_master_assay/2021.

[27] 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.html/2021.

[28] 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.

[29] W. Kami, T. Kinjo, W. Arakaki, H. Oki, D. Motooka, S. Nakamura, 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/.

[30] M. Sandoval Torrientes, C. Castello Abietar, J. Boga Riveiro, M.E. Álvarez-Argüelles, S. Rojo-Alba, F. Abdou Sibai, 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: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC7989071/.

[31] 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.bmj.com/lookup/doi/10.1136/bmj.n1445.

[32] 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/S138665392100144X.

[33] M. Sandoval Torrientes, C. Castello Abietar, J. Boga Riveiro, M.E. Álvarez-Argüelles, S. Rojo-Alba, F. Abdou Sibai, 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: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC7989071/.

[34] H. Wang, J.A. Miller, M. Verghese, M. Sibai, D. Solis, K.O. Mfuh, et al., Multiplex quantitative real-time 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.00741-21.

[35] Y. Zhao, A. Lee, K. Composto, M.H. Cunningham, J.R. Mediavilla, S. Fennessey, 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. (2021). 59 (4) [cited 2021 Jun 4] Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7901269/.

[36] Seegene. NovaplexTM SARS-CoV-2 variants 1 assay (RUO). 2021.

[37] E. M. Pinto, M. A. Pinto, S. Pinto, C. Pinto, J. Pinto, A. Pinto, et al., Evidence of escape of SARS-CoV-2, Variant with L452R and E484Q neutralization resistance mutations [Internet], J. Clin. Microbiol. (2021) [cited Aug 18]. Available from: https://journals.asm.org/doi/abs/10.1128/JCM.00741-21.

[38] G. Cabeceiras, 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.