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Evolving strategy for an evolving virus: Development of real-time PCR assays for detecting all SARS-CoV-2 variants of concern

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ABSTRACT

In order to detect the SARS-CoV-2 variants of concern (VOCs), five real-time reverse transcriptase PCR (rRT-PCR) assays were designed to target the critical discriminatory mutations responsible for the following amino acid changes in the spike protein: two Δ69–70 + N501Y + E gene triplexes (one optimized for Alpha [B.1.1.7] and one optimized for Omicron [B.1.1.529]), a ΔK417N + 242–244 wild-type duplex, a K417T + E484K duplex, and a L452R + P681 + E484Q triplex. Depending on the assay, sensitivity was 98.97–100% for the detection of known VOC-positive samples, specificity was 97.2–100%, limit of detection was 2–116 copies/reaction, intra- and interassay variability was less than 5%, and no cross-reactivity with common respiratory pathogens was observed with any assay. A subset of rRT-PCR-positive VOC samples were further characterized by genome sequencing. A comparison of the lineage designation by the VOC rRT-PCR assays and genome sequencing for the detection of the Alpha, Beta, Gamma, Delta and Omicron variants showed clinical sensitivities of 99.97–100 %, clinical specificities of 99.6–100 %, positive predictive values of 99.8–100%, and negative predictive values of 99.98–100 %. We have implemented these rRT-PCR assays targeting discriminatory single nucleotide polymorphisms for ongoing VOC screening of SARS-CoV-2 positive samples for surveillance purposes. This has proven extremely useful in providing close to real-time molecular surveillance to monitor the emergence of Alpha, the replacement of Alpha by Delta, and the replacement of Delta by Omicron. While the design, validation and implementation of the variant specific PCR targets is an ever-evolving approach, we find the turn-around-time, high throughput and sensitivity to be a useful complementary approach for SARS-CoV-2 genome sequencing for surveillance purposes in the province of Alberta, Canada.

1. Introduction

The World Health Organization (WHO) case definition for SARS-CoV-2 variants of concern (VOCs) includes lineages with increased transmissibility or detrimental change in COVID-19 epidemiology, increased virulence or change in clinical disease presentation, or decreased effectiveness of public health and social measures or available diagnostics, vaccines, and therapeutics (World Health Organization, 2021). As VOCs continue to emerge, rapid detection combined with the implementation of public health measures are imperative to control their spread. The current lineages declared by the WHO as VOCs include: Alpha (B.1.1.7), which was first documented in the United Kingdom in September 2020 and declared as a VOC on December 18, 2020 (Public Health England, 2020); Beta (B.1.351), which was first documented in South Africa in May 2020 and was declared as a VOC on December 18, 2020 (Tegally et al., 2021); Gamma (P.1), which was first detected in Brazil in November 2020 and declared as a VOC on January 11, 2021 (Faria et al., 2021); Delta (B.1.617.2), which was initially detected in
India in October 2020 and declared as a VOC on May 11, 2021 (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/, accessed March 17, 2022); and Omicron (B.1.1.529), which was first reported in multiple countries in southern Africa in November 2021 and declared as a VOC on November 26, 2021 (World Health Organization, 2021a; Viana et al., 2022). Based on the WHO epidemiological update on March 30, 2021, Alpha had been reported in 130 countries, Beta in 80 countries, and Gamma in 45 (World Health Organization, 2021c) countries; the spread of Delta was faster than prior VOCs and it was reported in 54 countries by June 1, 2021 (World Health Organization, 2021b). The rate of spread for Omicron surpassed all previous VOCs and as of Jan 6, 2022, Omicron had been identified in 149 countries across all six WHO Regions (World Health Organization, 2022).

Alpha has 13 mutations in the spike (S) protein with the Δ69–70, N501Y and P681H mutations contributing to its increased transmissibility (Davies et al., 2021). Gamma displays additional critical mutations including L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, and H655Y (amino acids located in the receptor-binding, N-terminal or furin cleavage domains of the spike protein), and has been shown to be more resistant to multiple neutralizing monoclonal antibodies and vaccine antibodies (Wang et al., 2021a). Beta also displays mutations at the same three receptor-binding domain residues as Gamma with K417N, E484K, and N501Y, which could reduce monoclonal antibody and vaccine efficacy (Tegally et al., 2021; Wang et al., 2021b). These residues are also associated with increased affinity to the human ACE2 receptor that can impact host cell entry and virus transmission (Lan et al., 2020). Important changes in the spike protein for Gamma with K417N, E484K, and N501Y, Δ70, Δ157, Δ158, L452R, D614G, P681R, and D950N (ECDC, 2021); L452R and E484Q can cause enhancement of human ACE2 receptor that can impact host cell entry and virus transmission (4.4 million) is carried out for symptomatic patients and, in some cases, asymptomatic patients who are close contacts of cases or involved in outbreaks. Upper respiratory tract samples included nasopharyngeal (NP) aspirates, throat or NP swabs collected and transported in Universal Transport Medium (COPAN Diagnostics (Murrieta, CA), Remel™ (Lenexa, KS), Yocon Biology (Beijing, China), Phoenix Airmid Biomedical (Ontario, Canada)), 0.85% saline (Djualyn Biologicals (Calgary, Canada)), or modified liquid Amies (COPAN Diagnostics). Lower respiratory tract samples included sputa, bronchoscopy specimens, or endotracheal tube suction. Testing data from April 3, 2021, to Jan 15, 2022, was included in this study. When resources were available, all SARS-CoV-2 positive samples were tested for the VOCs based on the number of cases and testing capacity. During the interim periods from May 1–31, 2021, September 10–November 24, 2021, and December 24, 2021–January 15, 2022, only targeted populations including patients involved in outbreaks, hospitalized/emergency room cases, inbound international travelers, and healthcare workers were screened because of increased numbers of positive cases and strain on available resources. As of April 3, 2021, all samples were screened for Alpha, Beta and Gamma lineages. Assays for the detection of SARS-CoV-2 and Alpha were multiplexed and this modification was implemented on April 14, 2021. Screening assay for the detection of the Delta variant was implemented on June 1, 2021, this assay was subsequently modified to include the E484Q target for the specific detection of B.1.617.2 on July 19, 2021. With the detection of the Omicron variant in late November 2021, it was noted that there were mutations for this lineage in our Δ69–70 deletion detection probe, N501Y forward primer and N501Y probe. As a result, these nucleotide positions were modified to accommodate for the detection of Omicron and a new triplex assay incorporating the modified Δ69–70 and N501Y oligonucleotides and the E gene marker was implemented on Dec 13, 2021 to replace the previous Δ69–70 + N501Y + E gene triplex. Fig. 1 shows the timeline for the implementation of the different VOC assays. This study was approved by the University of Alberta Human Research Ethics Board (reference number Pro00108722).

2.2. Assays for SARS-CoV-2 VOC screening and interpretation of VOC assays

Five assays incorporating 10 separate markers were designed during the time period of this study. A triplex assay incorporating previously reported primers and probes to detect N501Y, Δ69–70, and an E gene target was developed to test all samples for Alpha (the E gene target acted as a surrogate marker of viral load in a sample, as the VOC assays are reliable only for samples of sufficient viral load); this assay is referred to as the N501Y + Δ69–70 + E gene (Alpha) assay (Zelaya et al., 2021; Pabbaraju et al., 2021). Two duplex assays to detect 242–244 WT + K417N and E484K + K417T were also devised to detect Beta and Gamma as a second step for samples that resulted N501Y positive but Δ69–70 negative by the N501Y + Δ69–70 + E gene (Alpha) assay. Finally, a duplex assay for B.1.617 (detecting L452R + P681 WT) was developed as another second step in the testing process for samples negative for N501Y, once this parent lineage was recognized as significant. Conversion of this assay into a triplex (L452R + P681 WT + E484Q) was undertaken when B.1.617.2 (Delta) was recognized as a VOC rather than B.1.617.

If the defined constellation of mutations was detected by the variant assays, the sample was reported as belonging to the corresponding specific lineage. If the complete set of defining mutations was not detected or if additional changes were noted, the results were reported as “presumptive variant” and genome sequencing was performed for lineage confirmation. With the arrival of the Omicron variant in November 2021, the N501Y + Δ69–70 + E gene (Alpha) assay was modified for the detection of this variant (hence referred to as the N501Y + Δ69–70 + E gene [Omicron] assay) and was run alongside the 242–244 WT + K417N assay to identify Omicron-positive samples (those that were positive for all five markers). The E484K + K417T assay was discontinued since neither Beta nor Gamma were circulating in Alberta by that time. The N501Y + Δ69–70 + E gene (Omicron) assay was implemented on December 13, 2021, subsequent to which only Omicron and Delta were reported as VOCs and samples with all other
mutation patterns were subjected to genome sequencing. See Fig. 2 for a summary of how the VOC assays were interpreted based on the evolution of VOCs.

All primers and probes used are summarized in Table 1. These were purchased either from Applied Biosystems (ABI, Foster City, California) or from LGC Biosearch Technologies (Petaluma, CA, USA). The VOC assays were performed using TaqMan® Fast Virus One-Step RT-PCR Master Mix (ABI) with the following primer/probe concentrations in µM: E484K and E484Q = 0.4/0.08; K417T = 0.5/0.08; 242–244 WT, K417N, L452R, E gene, Δ69–70 (both Alpha and Omicron versions) = 0.8/0.2; P681 WT and N501Y (both Alpha and Omicron versions) = 0.8/0.07. Each reaction contained 5 µL of template nucleic acid. The reverse-transcription step was performed at 50 °C for 5 min followed by incubation at 95 °C for 20 s. Amplification included 45 cycles of denaturation at 95 °C for 3 s, followed by annealing, extension and data acquisition at 60 °C for 30 s on the 7500 Fast Real-Time PCR system (ABI).

2.3. Extraction of viral nucleic acid

Viral RNA from the different specimen types was extracted on one of two platforms according to manufacturers’ instructions: easyMAG® (BioMerieux, Quebec, Canada) with associated reagents or the MagMAX Express 96 or KingFisher Flex automated extraction and purification systems (Thermo Fisher Scientific) with either the MagMAX™-96 Viral RNA Isolation Kit (ABI) or the LuminUltra RNA Isolation Kit (LuminUltra Technologies Ltd., New Brunswick, Canada) in combination with the MagDx® AQM magnetic beads from Applied Quantum Materials Inc. (Alberta, Canada). The validated specimen types included throat swab, nasal swab, NP swab and aspirate, auger suction, bronchoalveolar lavage, endotracheal secretion, and lung tissue. The sample input and output volumes were 200 µL and 110 µL for all the respiratory sample types, and 60 µL and 200 µL for the tissue samples, respectively.

2.4. VOC assay analytical sensitivity/specificity, reproducibility, and accuracy

Regions of the spike gene including the targets for the VOC assays were PCR-amplified and cloned into a vector using the TOPO® TA Cloning Dual Promoter Kit (Life Technologies, California, USA). The plasmid DNA was linearized using restriction enzymes and the T7 RiboMAX™ Express (Promega, Madison, WI, USA) or RiboMAX™ SP6 RNA Production System (Promega, Madison, WI, USA) were used for the transcription of the plasmid DNA to generate in vitro RNA. The transcribed RNA was spectrophotometrically quantified for the calculation of copy numbers. The analytical sensitivity for the assay was determined by testing ten-fold serial dilutions of quantified in-vitro RNA in triplicate on three independent runs. The 95% limits of detection (95% LOD) were

![Fig. 1. Timeline for implementation of the VOC assays.](image1)

![Fig. 2. Interpretation of VOC assays.](image2)
Table 1

| Target | Primer/probe name | Primer/probe sequence (5’–3’) |
|--------|-------------------|--------------------------------|
| K417T  | K417N For         | ATGAGTGCAGCAAACTGCTCAC         |
|        | K417N Rev         | AAGCAGGCTGAAAGATCCTG           |
|        | K417T Probe       | VIC/AACCTGGAAGCTGTGGTAT/MGB-NFQ |
| E484K  | E484K For         | CACCTGGAGGGAGGGATGCA           |
|        | E484K Rev         | GCTGTCGTAGTAGGTCGTA            |
|        | E484K Probe       | FAM/CTTGTATAGGTTGAAAGGTTTAATT/BHQ1 |
| E484Q  | E484Q For         | CTACAGGGCGGTGACGA              |
|        | E484Q Rev         | GCTGTCGTAGGAAGGTCGTA           |
|        | E484Q Probe       | NED/CTTGTATAGGTTGAAAGGTTTAATT |
| K417N  | K417N For         | ATGAGTGCAGCAAACTGCTCAC         |
|        | K417N Rev         | AAGCAGGCTGAAAGATCCTG           |
|        | K417N Probe       | VIC/AACCTGGAAGCTGTGGTAT/MGB-NFQ |
| 242–44 deletion | 242/4 For         | GGTCTTAAACCTTGGTAGATTTG        |
|        | 242/4 Rev         | CCGGAGAAGGATACCGGAGGCT         |
|        | 242/4 WT Probe    | FAM/ACACCTTGGTAGGCCAGT/MGB-NFQ |
| L542R  | L542R For         | TAAACATCTGAGTACTAAGGCTTG       |
|        | L542R Rev         | TACCGGCGCTGATGATTCTGGT         |
|        | L542R Probe       | VIC/AATTTAACTACGGGTAATAGTG/TQY |
| P681   | P681H For         | GGTATATCCTCGAGTATACGACTCG      |
|        | P681H Rev         | ATGATGAGTGGACTGACATG           |
|        | P681H WT Probe    | FAM/ACTAATTCCTCCTGGCGG/MGB-NFQ |
| 69–70 deletion | 69–70 Rev         | GACGGAGTTCACCACTACCTGAGACC     |
|        | 69–70 Del Probe   | VIC/CATGGTACTCCCTGGG/MGB-NFQ   |
| N501Y  | N501Y For         | GAAGGTTTGAATTGGACTTCTCC        |
|        | N501Y Rev         | AACCATGTTCTGGATGCTCGA          |
|        | N501Y Probe       | FAM/CAACACCTACTGGTGTT/BHQ1     |
|        | N501Y1           | FAM/CRACACCTACTGGTGTT/BHQ1     |
| E-gene | E-For V2          | GGAGACGATCGATTAATGGTTTACG      |
|        | E-Rev V2          | CAAATGAGGACGATGTAAGAGCAC       |
|        | E-Probe          | NED/CTAGGCGGAATCTCGAG/MGB-NFQ  |

* Primers and probes for N501Y were designed by Ontario Agency for Health Protection and Promotion (PHOL, Ontario, Canada, (Abdulnoor et al., 2022)), the E484K assay was developed at PHOL by Alireza Esbaghi (publication pending) and shared with Alberta Precision Laboratories. All other primers and probes were designed in-house.

Calculated by probit analysis. Analytical specificity (cross-reactivity) of each assay was determined by testing a panel of pathogens which included coronaviruses (NL63, OC43, 229E, HKU1, MERS-CoV, SARS-CoV-1), influenza A H3N2, influenza B, respiratory syncytial virus, parainfluenza virus, rhinovirus 1b, enteroviruses (cox sackievirus B6), adenovirus, bocavirus, human metapneumovirus, Streptococcus pneumoniae, Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumophila, Bordetella pertussis, Haemophilus influenzae, and Neisseria meningitidis. The intra- and inter-assay variability was determined using high (Ct value of ~20) and low (Ct value of ~30) viral load samples for the Alpha, Beta, Gamma, Delta and Omicron lineages, with all samples being tested in triplicate on three independent runs.

To assess the accuracy of detection of the Alpha, Beta, Gamma, Delta and Omicron lineages, a panel of samples positive for the mentioned lineages and wild-type non-VOC lineages were tested by all assays. The lineages were identified based on whole-genome sequence analysis carried out at Alberta Precision Laboratories – Provincial Public Health Laboratory (ProvLab). A total of 77 Alpha-positive and 148 Alpha-negative samples were tested by the Δ69–70 + N501Y + E-gene (Alpha) assay, 16 Beta-positive and 72 Beta-negative samples were tested by the 242–44 WT + K417N assay, 18 Gamma-positive and 70 Gamma-negative samples were tested by the K417T + E484K assay; 20 Delta-positive and 75 Delta-negative samples were tested by the P681H WT + L542R + E484Q assay, and 24 Omicron-positive and 136 Omicron-negative were tested by the Δ69–70 + N501Y + E-gene (Omicron) assay for validation.

2.5. Genome sequencing (GS) and prospective comparison with the VOC assays

The full genome of SARS-CoV-2 was amplified by multiplex PCR using the Freedom primer scheme (Freed et al., 2020) as 1200 bp amplicons or the Resende primer scheme as 2000 bp amplicons (Resende et al., 2020). The Nanopore libraries were prepared using the ARTIC LoCost protocol (Tyson et al., 2020) and the Ligation sequencing kit (SQK-LSK109) where 15–20 ng of the library was loaded on the FLO-MIN 106D flow cells from Oxford Nanopore Technologies (ONT). Alternatively, Illumina libraries were made using the DNA Prep Kit (Illumina), and sequenced on an Illumina MiSeq or MiniSeq using the 300 cycle MiSeq Reagent Kit V2 Micro or MiniSeq Mid Output Cycle Kit respectively (Illumina).

Consensus genomes from data generated with ONT were compiled through the artic 1.1.3 pipeline (https://github.com/artic-network/fi eldbiobinformatics). The Illumina data was processed with the OICR fork (https://github.com/oicr-gsi/nov2019-artic-nl) of the nov2019-illumina-nf pipeline (https://github.com/connor-lab/nov2019-artic-nl), this pipeline was further updated to use freebayes as the variant caller (https://github.com/jts/nov2019-artic-nf). The quality of the sequencing runs was assessed novqc (https://github.com/jts/novcomm-tools); pangolin was used to assign lineages (https://www.nature.com/articles/s41564-020-0770-5) and nextclade was used to detect mutations (github.com/nexstrain/nextclade).

A subset of COVID-19 positive samples tested by the VOC assays underwent GS for lineage determination and VOC confirmation. This permitted the calculations of clinical sensitivity, clinical specificity, positive predictive value (PPV), and negative predictive value (NPV) for the VOC assays in comparison to WGS.

2.6. Statistical analysis

Accuracies of the VOC assays were calculated as (true positives + true negatives)/(true positives + true negatives + false positives + false negatives) × 100%. Inter- and intra-assay variability for the VOC assays were determined by calculating and percent coefficients of variation (% CV) using Ct values for high and low viral load samples run as replicates.

Seven-day rolling averages were determined for specimens testing positive for any of the VOCs using the VOC assays. The percent positivity for each VOC was calculated as a proportion of these lineages to the total number of SARS-CoV-2 positive samples that were tested by the VOC assay each day including samples with and without successful screening results. Using genome sequencing as the reference method, clinical sensitivity (true positives/[true positives + false negatives] × 100%), clinical specificity (true negatives/[true negatives + false positives] × 100%), PPV (true positives/[true positives + false positives] × 100%), and NPV (true negatives/[true negatives + false negatives] × 100%) of the VOC assays were calculated.
Table 2

Performance of the SARS-CoV-2 variant assays.

| Strain or in-vitro RNA | ΔH69 | V70 N501Y E gene 242–244 | K417N | L452R | P681WT |
|------------------------|------|--------------------------|-------|-------|--------|
| Alpha/Beta/Gamma/      |      | -                        | -     | -     | -      |
| Analytical specificity | 100% | 100%                     | 100%  | 100%  | 100%   |
| Intra-assay reproducibility | 1.21 | 0.10                     | -     | -     | -      |
| Inter-assay reproducibility | 1.75 | 0.06                     | 1.92  | 0.10  | -      |
| Average Ct values      |       |                          | 3.52  | 0.22  | 3.86   |
| Sensitivity (95% CI)   |       |                          | 0.83  | 0.21  | 0.89   |

a Analytical specificity was based on cross-reactivity to 34 commonly found respiratory pathogens.
b Numbers of positive and negative samples used for the calculation of accuracy are outlined in the text.
c Sensitivity of detection was implemented on December 13, 2021, and the VOC assay for the detection of this lineage was implemented on December 13, 2021, when 20 % of the samples belonged to this lineage. A dramatic increase to greater than 80 % was noted by December 22, 2021. Currently there is almost exclusive circulation of Omicron in Alberta.
4. Discussion

Variants of concern pose a threat to the population because these strains can change the epidemiology in terms of transmission, severity of disease, vaccine efficacy and treatment options. Mutations in the spike (S) glycoprotein can alter the domain that recognizes the host cell ACE2 (angiotensin-converting enzyme 2) receptor and the targets for neutralizing antibodies. These mutations can also change the conformational B-cell epitopes leading to potential reductions in vaccine efficacy (Salleh et al., 2021). There is evidence that all VOCs are more transmissible than the wild-type virus (Curran et al., 2021; Campbell et al., 2021; Funk et al., 2021). A meta-analysis conducted on studies from June 1, 2020 to October 15, 2021 showed that Alpha, Beta, Gamma, and Delta cause more severe disease than the wild-type virus in terms of hospitalization, intensive care unit (ICU) admission, and mortality, and Beta and Delta carried a higher risk than Alpha and Gamma (Lin et al., 2021). Study of a large cohort in Ontario, Canada, showed that compared with the wild-type SARS-CoV-2 strains, the adjusted elevation in risk for hospitalization, ICU admission and death was higher for Alpha, Beta and Gamma, and the increased risk with Delta was even more pronounced (Fisman and Tuite, 2021).

Public health labs have needed to respond to the VOCs in a manner similar to the original SARS-CoV-2 by having the ability to detect and differentiate the VOCs in a high-throughput fashion with a quick turn-around-time. In response to these evolving variants, rRT-PCR assays targeting constellations of mutations characteristic for a particular VOC were designed, validated, and implemented in our jurisdiction for testing high volumes of specimens that were positive for SARS-CoV-2. The VOC testing and reporting algorithm evolved as the number of cases and prevalence of different VOCs fluctuated in our population. When the number of cases was low, all positive samples were tested for VOCs; however, when the cases were high, laboratories were under...
intense pressure to focus on performing high volumes of diagnostic COVID-19 testing and thus VOC testing was narrowed to only certain populations where it was deemed to be of a higher impact. This real-time surveillance of VOCs was used by public health personnel to control the spread of the virus in multiple settings, such as outbreaks in schools, congregate living facilities, and hospitals. After the initial detection of VOCs by the rRT-PCR assays, a proportion of the circulating VOCs and non-VOCs were further characterized by genome sequencing for surveillance. This allowed the monitoring of VOC sub-lineages if present and a comparison of the sequences circulating in our community to the globally circulating lineages. This strategy was also designed to detect the arrival of new lineages by preferentially characterizing all the non-VOC lineages (as determined by the VOC assays) by genome sequencing. For example, once Delta became the predominant lineage, all the samples that tested as non-Delta were characterized by NGS and the same strategy is in current use with the dominance of Omicron.

Comparison of the lineage designation using the VOC assays to genome sequencing shows that the VOC assays provide reliable results but with much faster turnaround time with suitable clinical sensitivity and specificity. An additional benefit of rRT-PCR assays is that samples with lower viral load that cannot be characterized by genome sequencing can be successfully tested for VOCs. Full genome sequencing of a significant proportion of samples that are interpreted as non-VOCs by the VOC assays also allows the detection of nucleotide changes in the primer and probe binding regions that could result in false-negative results by the VOC assays. As outlined in the results, four samples showed the C21709T mutation in the Δ69–70 probe, two samples showed G23069T in the N501Y probe and two samples had G22918A and T22917A changes in the I452R probe leading to false-negative results. This combined use of rRT-PCR assays and genome sequencing represents a powerful approach whereby large numbers of samples can have their VOC status determined rapidly while simultaneously prioritizing samples for a more complete characterization when they may represent an emerging lineage of medical and public health importance. This approach also focuses genome sequencing resources on high-priority samples so that reagents and labor are not needlessly used to sequence samples that can be genotyped using more routine rRT-PCR reagents and resources.

As reported in the literature, we noted limited transmission of the Beta lineage in Alberta, with increased spread of the Gamma lineage in comparison. The enhanced transmissibility of the Delta and Omicron lineages was evident with the sharp rise and near-complete dominance with the highest number of analyzed sequences for the five VOCs showed that the transmissibility was highest for the omicron variant followed by Delta, Alpha, Gamma and Beta respectively. The highest estimated growth rates and reproduction numbers were due to the Omicron variant indicating the highest transmissibility (Mananthisa et al., 2022). Data from another Canadian province of Ontario indicates similar trends in the rise of VOCs, after the initial detection of B.1.17 in Dec 2020, and increase to more than 90 % was noted (Tuitte et al., 2021) and an increase for Delta from 2.2 % in early April to 83 % in late May was noted (Brown et al., 2021). Estimates of VOC prevalence between Mar 1, 2021 to Nov 15, 2021 in Ontario show that 99.3 % of COVID-19 were of the Delta variant, 0.2 % of the cases were estimated to be Alpha and 0.2 % were estimated to be Beta, Gamma or Mu (Ontario, 2021).

The implementation of high throughput variant screening assays promptly after the identification of VOCs worldwide, has helped to guide public health policies. Information on circulating VOCs by the screening assays has helped to prioritize samples for genome sequencing in our population resulting in optimal use of genome sequencing resources and robust surveillance of circulating genotypes and emerging trends to monitor the evolution of SARS-CoV-2 in the province. Thus the provincial strategy adopted was a combined approach to rapidly detect known VOCs while continuously monitoring for evolving mutations.

Declaration of Competing Interest

The authors do not have any financial or personal conflicts of interest to declare.

Data availability

Data will be made available on request.

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References

Abdulnoor, M., Esghahi, A., Perusini, S.J., Broukhanski, G., Corbeil, A., Cronin, K., et al., 2022. Real-Time RT-PCR allelic discrimination assay for detection of N501Y mutation in the spike protein of SARS-CoV-2 associated with B.1.1.7 variant of concern. Microbiol. Spectr. 10 (1), e0068121.
Augusto, G., Mohnen, M.O., Zinkahn, S., Liu, X., Vogel, M., Bachmann, M.F., 2022. In vitro data suggest that Indian delta variant B.1.617 of SARS-CoV-2 escapes neutralization by both receptor affinity and immune evasion. Allergy 77 (1), 111–117.
Brown K.A., Joff E., Buchan S.A., Daneman N., Mishra S., Patel S., et al., 2021. Infection in prevalence of SARS-CoV-2 infections missing the N501Y mutation as a marker of rapid Delta (B.1.617.2) lineage expansion in Ontario, Canada. medRxiv. 2021.06.22.21209349.
Campbell, F., Archer, B., Laurensen-Schaffer, H., Jinnai, Y., Konings, F., Batra, N., 2021. Increased transmissibility and global spread of SARS-CoV-2 variants of concern as of June 2021. Eur. Surveill. 26 (24).
Curns, J., Del, J., Boulus, L., Somerville, M., McCulloch, H., MacDonald, M., 2021. Transmission characteristics of SARS-CoV-2 variants of concern rapid scoping review. medRxiv. 2021.04.23.21215515.
Davies, N.G., Abbott, S., Barnard, R.C., Jarvis, C.I., Kucharski, A.J., Munday, J.D., 2021. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science 372 (6538), 6354–6355.
ECDC, 2021. Emergence of SARS-CoV-2 B.1.617 variants in India and situation in the EU/EEA, 11 May 2021.
Faria, N.E., Mellan, T.A., Whitaker, C., Claro, I.M., Candido, D.D.S., Mishra, S., 2021. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. Science 372 (6544), 815–821.
Fisman, D.N., Tuite, A.R., 2021. Evaluation of the relative virulence of novel SARS-CoV-2 variants: a retrospective cohort study in Ontario, Canada. Can. Med. Assoc. J. 193 (42), E1619–E1625.
Freed, N.E., Vikola, M., Faiyal, M.B., Silander, O.K., 2020. Rapid and inexpensive whole-genome sequencing of SARS-CoV-2 using 1200 bp tiled amplicons and Oxford nanopore rapid barcoding. Biol. Methods Protoc. 5 (1), 49014.
Funk, T., Pharris, A., Spiteri, G., Bundle, N., Melidou, A., Carr, M., 2021. Characteristics of SARS-CoV-2 variants of concern B.1.1.7, B.1.351 or P.1: data from seven EU/EEA countries, weeks 38/2020 to 10/2021. Eur. Surveill. 26 (16).
Gu, H., Krishnan, P., Ng, D.Y.M., Chang, L.D.J., Liu, G.Y.Z., Cheng, S.S.M., et al., 2022. Probable transmission of SARS-CoV-2 omicron variant in quarantine Hotel, Hong Kong, China, November 2021. Emerg. Infect. Dis. 28 (2), 460–462.
Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., et al., 2020. Structure of the SARS-CoV-2 spike receptor-binding domain binding to the ACE2 receptor. Nature 581 (7807), 215–226.
Lin, L., Liu, Y., Tang, X., He, D., 2021. The disease severity and clinical outcomes of the SARS-CoV-2 variants of concern. Front. Public Health 9, 775224.
Mananthisa, S.S., Abeygunawardena, I.A., Dharmanarte, S.D., 2022. Viral. J Matic, N., Lowe, C.F., Ritchie, G., Stefanovic, A., Lawson, T., Jang, W., et al., 2021. Rapid detection of SARS-CoV-2 variants of concern, including B.1.1.28/P.1, British Columbia, Canada. Emerg. Infect. Dis. 27 (6), 1675–1676.
Ontario, 2021. Estimating the prevalence and growth of SARS-CoV-2 variants in Ontario using mutation profiles. [Available from: https://www.publichealthontario.ca/-/media/documents/nocv/epi/covid-19-prevalence-growth-voc-mutations-epi-summary.pdf?la=en].
Ozono, S., Zhang, Y., Ode, H., Sano, K., Tan, T.S., Imai, K., et al., 2021. SARS-CoV-2 D614G spike mutation increases entry efficiency with enhanced ACE2-binding affinity. Nat. Commun. 12 (1), 484.
Pabbaraju, K., Wong, A.A., Douzenard, M., Ma, R., Gill, K., Dien, P., et al., 2021. Development and validation of RT-PCR assays for testing for SARS-CoV-2. Off. J. Assoc. Med. Microbiol. Infect. Dis. Can. 6 (1), 16–22.
Phan, T., Boes, M., McCullah, M., Gribesch, J., Marsh, J., Harrison, L.H., et al., 2022. Development of a one-step competitive RT-PCR assay to detect the SARS-CoV-2 omicron (B.1.1.529) variant in respiratory specimens. J. Clin. Microbiol. 60 (3), e002422.
Public Health England, 2020. Investigation of novel SARS-COV-2 variant of concern 202012/01 [updated Dec 21, 2020. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/959438/Technical_Briefing_VOC_SH_NJL2_SH2.pdf].

Resende, P.C., Motta, F.C., Roy, S., Appolinario, L., Fabri, A., Xavier, J., et al., 2020. SARS-CoV-2 genomes recovered by long amplicon tiling multiplex approach using nanopore sequencing and applicable to other sequencing platforms. bioRxiv, 2020.04.30.069039.

Salleh, M.Z., Derrick, J.P., Deris, Z.Z., 2021. Structural evaluation of the spike glycoprotein variants on SARS-CoV-2 transmission and immune evasion. Int J. Mol. Sci. 22 (14).

Takemae, N., Doan, Y.H., Momose, F., Saito, T., Kageyama, T., 2022. Development of new SNP genotyping assays to discriminate the Omicron variant of SARS-CoV-2. Jpn J. Infect. Dis.

Tegally, H., Wilkinson, E., Giovanetti, M., Iranzadeh, A., Fonseca, V., Giandhari, J., 2021. Detection of a SARS-CoV-2 variant of concern in South Africa. Nature 592 (7854), 438–443.

Tuite A.R.F.D., Odutayo A., et al., 2021. COVID-19 hospitalizations, ICU admissions and deaths associated with the new variants of concern. Science Briefs of the Ontario COVID-19 Science Advisory Table [1 (8):Available from: https://doi.org/10.47526/ocsat.2021.02.18.1.0.

Tyson, J.R., James, P., Stoddart, O., Sparks, N., Wickenhagen, A., Hall, G., et al., 2020. Improvements to the ARTIC multiplex PCR method for SARS-CoV-2 genome sequencing using nanopore. bioRxiv, 2020.09.04.283077.

Vega-Magana, N., Sanchez-Sanchez, R., Hernandez-Bello, J., Venancio-Landeros, A.A., Pena-Rodriguez, M., Vega-Zepeda, R.A., et al., 2021. RT-qPCR assays for rapid detection of the N501Y, 69-70del, K417N, and E484K SARS-CoV-2 mutations: a screening strategy to identify variants with clinical impact. Front. Cell Infect. Microbiol. 11, 672562.

Viana, R., Moyo, S., Amoako, D.G., Tegally, H., Schepers, C., Althaus, C.L., et al., 2022. Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. Nature.