Rapid, cost-effective system for Surveillance of SARS-CoV-2 variants of concern using targeted RT-PCR SARS CoV-2 mutation panel

N.Pinkhover[1], E.Sanchez[1], K.Pontbriand[1], J.Auclair[1], M.Gandhi[2], S.Vishnubhotla[2], T.Proctor[2],
1 Life Science Testing Center, Northeastern University, Burlington, MA, USA, 2 Thermo Fisher Scientific, South San Francisco, CA, USA

INTRODUCTION

As COVID-19 vaccination rates remain unsatisfactory, the exponential spread of SARS-CoV-2 variants of concern continue to pose a threat to global public health. Surveillance testing is critical for tracking viral progression, vaccine breakthrough, and emerging SARS-CoV-2 variants as the pandemic continues to evolve. While whole-genome sequencing (WGS) is the primary method used for genomic surveillance, it is a costly and resource-intensive process for implementation across population scale. In this study, we describe an alternative, lower-cost quick PCR-based method for the detection and discrimination of 4 currently designated VOCs; alpha (B.1.1.7), beta (B.1.351), gamma (P.1), and delta (B.1.617).

EXPERIMENTAL METHODOLOGY

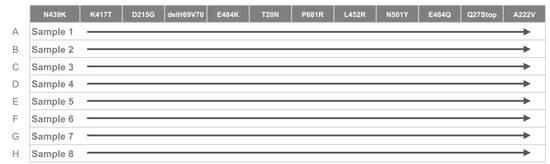
The study included 108 SARS-CoV-2 positive specimens received at the Life Science Testing Center at Northeastern University between April to August 2021. RNA was extracted using Applied Biosystems™ MagMax™ Viral/Pathogen II Nucleic Acid Isolation Kit automated on Agilent™ Bravo Liquid Handlers and SARS-CoV-2 status was determined using Applied Biosystems™ TaqPath™ COVID-19 Combo Kit. Variant profiling was performed on extracted RNA using a panel of TaqMan SARS-CoV-2 genotyping assays. 5.0 µL of sample RNA or Thermo Scientific™ Acrometrix™ Coronavirus 2019 (COVID-19) RNA Control was then aliquoted into 96-well optical plates containing TaqMan™ SARS-CoV-2 variant-specific assay mix (15µL) as shown in Table 1.

Table 1. Volumes of each component for 96-well plate Applied Biosystems™ TaqMan™ SARS-CoV- 2 Mutation Panel Setup.

Component	Volume per assay	Volume per 96-well plate
TaqPath™ 1-Step RT-qPCR Master Mix, CG (4X)	5 µL	528 µL
TaqMan™ SARS-Cov-2 Mutation Panel Assay (40X)	0.5 µL	52.8 µL
Nuclease-free Water	9.5 µL	1003.2 µL
Total Reaction Mix Volume	15 µL	1584 µL

Both a 12- and 8-assay panel were tested using the following assays: N439K, K417T, D215G, delH69/V70, E484K, T20N, P681R, L452R, N501Y, E484Q, Q27STOP, A222V. The 8-assay panel excluded the N501Y, E484Q, Q27STOP, and A222V assays. 44 samples were run using the 8-assay panel and 64 using the 12-assay panel. Layout of 8 samples on the 96-well optical plate for the 12-assay panel is illustrated in Figure 1.

Figure 1. 96-well optical plate layout



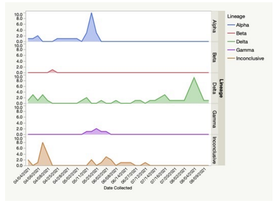
The SARS-CoV-2 lineages were assigned using a classifier based on the MOC combination (Table 2). Initial mutant results and variant classification were verified by next-generation sequencing using Ion Torrent™ GeneStudio™ S5 Plus System. Linages were assigned based on the MOC data analysis that was performed using Applied Biosystems™ Design and Analysis Software Version 2.5.1, via allelic discrimination scatter plots for variant genotyping determination. Further statistical analyses and graphical data representations were performed using JMP® Pro 15.0.0.

Table 2. 12 MOC-panel for VOC discrimination. Note: E484Q (B.1.617.1 and B.1.617.3) and A222V (B.1.177) were included in the panel to cover other variants.

SARS-CoV-2 Lineage	N439K	K417T	D215G	delH69/V70	E484K	T20N	P681R	L452R	N501Y	E484Q	Q27Stop	A222V
Alpha (B.1.1.7)	Y			Y					Y		Y	
Beta (B.1.351)			Y		Y				Y			
Gamma (P.1)		Y			Y	Y			Y			
Delta (B.1.617.2)							Y	Y				

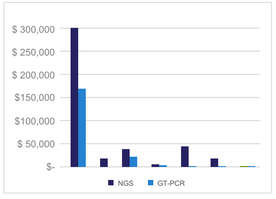
RESULTS

Figure 2. Frequency of SARS-CoV-2 lineages in screened positive patient samples at Northeastern University from April to August 2021 using PCR-based MOC panel.



Of the 108 samples, 25 samples were classified as Alpha variant, 1 as Beta, 7 as Gamma, and 48 as Delta. SARS-CoV-2 linages could not be assigned for 27 samples (Figure 2).

Figure 3. Cost benefit analysis between NGS and MOC panel for VOC surveillance testing.

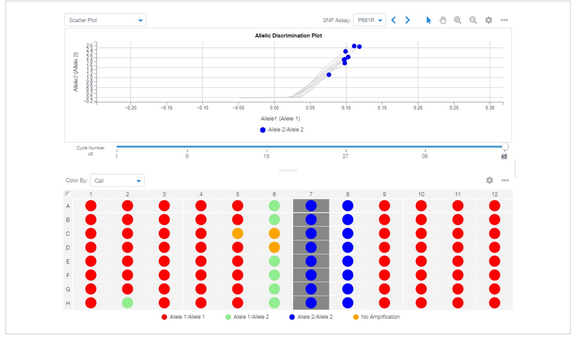


	Instrument Cost	CPU/GPU Cost	Kit/Reagent Cost	Staff Cost	Initial 96-Sample run	Optimized 96-Sample Run	Cost Per Sample
NGS	$303,365	$13,790	$39,820	$2,760	$42,580	$21,287	$81
GT-PCR	$168,100	$0	$19,700	$1,380	$304	$1,969	$21

The MOC-panel is nearly 4X cheaper than sequencing with a per sample cost of $20.50 for 96 samples (MOC panel) as compared to $81.39 (NGS) (Figure 4).

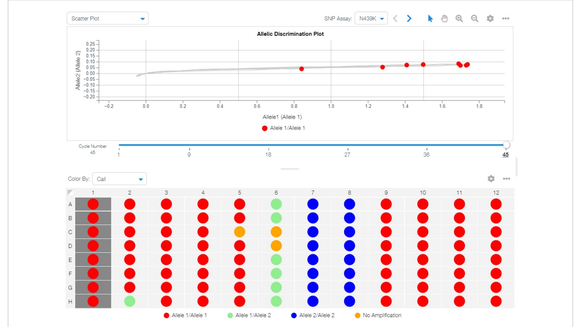
12-Assay MOC Panel confirmation of B.1.617.2 (Delta- specific) P681R SARS-CoV-2 mutation in 8 samples collected from NU population is shown in Figure 3.

Figure 4. Eight (8) confirmed Delta variants displaying full amplification of the Proline681Arginine (P681R) spike glycoprotein mutation using the 12-assay mutant panel. Samples expressing VIC–labeled mutant alleles (blue) amplify linearly in the y-axis.



12-assay MOC panel confirming absence of B.1.258 (multi-variant) N439K SARS-CoV-2 mutation in 8 samples Collected from NU Population. Column-4 and Column-11 displaying WT/reference amplification for 2 B.1.1.7 (Alpha-specific) del.H69V70 and Q27STOP SNPs across the same 8 NU samples seen in Figure 4.

Figure 5. The same eight (8) samples from figure 2 displaying reference allele (WT) amplification of the Asparigine439Lysine (N439K) of the spike glycoprotein. Samples expressing WT alleles (FAM-labeled) clustered linearly along the x-axis.



- The 8-assay panel identified variant class with a mean of 45.50% (n = 20/44, p = 0.05).
- The 12-assay panel layout identified variant class with a mean of 95.31% (61/64, *p* = 0.05).
- Used in combination, the 8-assay and 12-assay MOC panels conclusively identified four (4) current VOCs (Alpha, Beta, Delta, Gamma) with a mean of 75.0% (n = 81/104, *p* = 0.05) of previously confirmed positive COVID-19 samples.
- Despite a negligible cost difference between layouts (the slight cost advantage for the 8-assay panel), the 12-assay layout showed a substantial increase in mean analytical performance.
- Comparison of the two plate layouts revealed the 12- assay layout was superior in performance by a factor >2.
- These data indicate our optimized 12-assay layout using the TaqMan™ SARS-CoV-2 Mutation Panel provides a significantly reliable, robust, and highly scalable method for surveillance of SARS-CoV-2 Variants of Concern.

CONCLUSIONS/DISCUSSION

SNP-PCR panels offer an easily implemented, cost- effective and robust method for at-scale surveillance of SARS-CoV-2 Variants of Concern. Optimizing 96-well MOC panels is critical for achieving high VOC classification and cost-benefit for large-scale surveillance. MOC panels afford efficient workflows and fast turn-around-times, which is crucial in monitoring the evolution of COVID-19 pandemic.