Rapid detection of SARS-CoV-2 variants of concern by single nucleotide polymorphism genotyping using TaqMan assays

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A R T I C L E   I N F O

A B S T R A C T

We evaluated the performance of SARS-CoV-2 TaqMan real-time reverse-transcription PCR (RT-qPCR) assays (ThermoFisher) for detecting 2 nonsynonymous spike protein mutations, E484K and N501Y. Assay accuracy was evaluated by whole genome sequencing (WGS). Residual nasopharyngeal SARS-CoV-2 positive samples ($N = 510$) from a diverse patient population in New York City submitted for routine SARS-CoV-2 testing during January-April 2020 were used. We detected 91 (18%) N501Y and 101 (20%) E484K variants. Four samples (0.8%) were positive for both variants. The assay had nearly perfect concordance with WGS in the validation subset, detecting B.1.1.7 and B.1.526 variants among others. Sensitivity and specificity ranged from 0.95 to 1.00. Positive and negative predictive values were 0.98−1.00. TaqMan genotyping successfully predicted the presence of B.1.1.7, but had significantly lower sensitivity, 62% (95% CI, 0.53, 0.71), for predicting B.1.526 sub-lineages lacking E484K. This approach is rapid and accurate for detecting SARS-CoV-2 variants and can be rapidly implemented in routine clinical setting.

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1. Introduction

The pandemic of COVID-19 caused by SARS-CoV-2 rapidly spread to other countries, including the United States. Since the SARS-CoV-2 virus emerged there have been multiple SARS-CoV-2 variants circulating globally [1]. While variants of SARS-CoV-2 are not new, there has been a drastic change in the number of mutations the virus has accumulated in short periods of time, causing concern around the world [2,3]. These variants range from having increased transmissibility or even potentially increased severity of disease and resistance to therapy and/or vaccine becoming an issue of significant public concern [4]. Since May 2021 the U.S. Center of Disease Control and Prevention (CDC) and the European Centre for Disease Prevention Control (ECDC) have identified numerous SARS-CoV-2 lineages as variants of concern or interest (VOC/VOI) [5]. These lineages all share certain spike proteins mutations, including E484K and N501Y. Of special concern were the B.1.1.7 (also 501Y.V1, Alpha) and the B.1.526 (Iota) lineages identified in the United Kingdom (UK) during the fall of 2020 and in New York City (NYC) in early 2021 [6,7], respectively.

These variants have been characterized by numerous distinct mutations in the spike protein [4,7]. The B.1.1.7 SARS-CoV-2 lineage is defined by 17 amino acid changes, including 8 changes in the spike protein (Rambaut et al., 2020). Of particular interest is the N501Y mutation in the receptor-binding domain of the spike protein. This mutation affects the receptor binding domain (RBD) of the spike protein, improving the virus' ability to enter human cells and increase infectivity. The B.1.526 lineage is defined by 16 amino acid changes, 7 of which are present in the spike protein [7] and include the E484K mutation. Other VOCs, B.1.351 (originating in South Africa) and P.1 (Brazil) [8,9] harbor both the N501Y and E484K mutations.

Routine sequencing of the SARS-CoV-2 genome from positive samples provides crucial insights into viral evolution and can support outbreak and surveillance analysis as well as clinical management [9,10]. Whole genome sequencing (WGS) is the best way to truly confirm the emergence of new strains of COVID-19. This involves analyzing the entire SARS-CoV-2 genome (at least 29,000 bp), making it much more complicated, time-consuming, and expensive than a PCR test [9,10]. It also requires special expertise.

In this study we have used targeted PCR assays to rapidly identify the N501Y and E484K mutations suggestive of the B.1.1.7 and B.1.526 SARS-CoV-2 variants in New York City during January−April 2020. We also performed orthogonal whole genome next generation

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sequencing (NGS) testing on a subset of samples to confirm these lineages.

2. Methods

2.1. Clinical specimens

The study used remnant sample from nasopharyngeal swabs in viral transport medium (NP-VTM) used in standard of care testing of patients with suspected COVID-19 infection. Positive SARS-CoV-2 specimens with a cycle threshold (C_T) value <30 were included. A total of 615 samples tested on the Roche 6800, Hologic Panther Fusion or rapid PCR testing systems (Roche LIAT and BioFire) were collected between January 1 and April 30, 2021, and used for the study (Fig. 1). The study was approved by Weill Cornell Medicine institutional review board (WCM-IRB).

2.2. TaqMan real time PCR

Extraction of total nucleic acid (TNA) from 200uL of NP-VTM samples was performed using an off-board lysis viral inactivation step followed by automated extraction of total nucleic acid (TNA) using the QIAsymphony DSP Virus/Pathogen Mini Kit coupled on the QIAsymphony SP (Qiagen, Germantown, MD) as previously described [10]. Positive control material was obtained from Twist Bioscience (San Francisco, CA). Two previously reported mutations in SARS-CoV-2 were selected for the analysis based on their association with numerous emergent lineages: N501Y (A23063T) and E484K (G2301A) in the spike gene. Genotyping analysis was performed using Custom TaqMan® Gene Expression Assays and the ABI Quant-Studio Real-Time PCR system (ThermoFisher Scientific, Waltham, MA) as described in the Supplemental Methods. Variant calls were assigned automatically based on the genotype-specific position of the spectral plots. Sequence variants were called "undetermined" if they clustered in proximity to the negative (water) controls (<1 fluorescence units), showed an abnormal spectral plot, or did not cluster tightly (outliers) (Fig. 2).
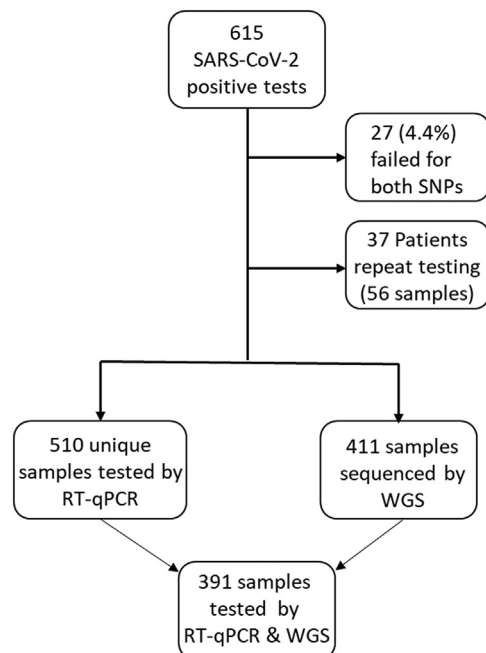
2.3. Whole genome sequencing (WGS)

Accuracy was validated by whole-genome sequencing (WGS) on a cohort of 395 NP-VTM specimens as described in the Supplemental Methods. Briefly, NP-VTM samples were extracted using the Mag-MAX Viral Pathogen Nucleic Acid Isolation kit on the KingFisher Flex Purification system (ThermoFisher). SARS-CoV-2 targeted assay libraries were prepared using Molecular Loop Viral RNA Target Capture Kit (Molecular Loop) according with manufacturer's recommendations. Libraries were then quantified and sequenced on a NovaSeq 6000 sequencer with 2 × 150bp reads. Following quality check, single end reads were mapped against the SARS-CoV-2 genome reference using BWA-MEM v0.7.17 [11]. Genome sequences were determined by molecule alignment pileup consensus calling with a minimum support of 5 molecules. SARS-CoV-2 lineage and clade classification were determined by Phylogenetic Assignment of Named Global Outbreak (pangolin, https://cov-lineages.org/resources/pangolin.html). PANGO lineage names were assigned by pangolin v2.4.1 and pango-LEARN June 5, 2021.

2.4. Statistical analysis

Continuous data were compared with ANOVA or Kruskal-Wallis, whereas categorical data were compared with Fisher exact test or $\chi 2$ test. Positive and negative predictive values (PPV, NPV) were reported with 95% confidence interval (CI), using NGS as the reference method. Statistical analyses were performed using R version 4.1.2 (November 1, 2021.)

3. Results

3.1. TaqMan real time PCR testing

During the period between January 2021 and April 2021 we evaluated 615 samples from 566 unique patients. Of those 27/566 (4.8%) failed for both SNPs (Fig. 1). The cycle threshold (Ct) values for the failed samples tended to be higher (Ct> 29) compared to the genotyped samples. Among the successfully genotyped samples, we identified 37 patients (56 samples) with repeated testing. No discordant results were observed. For standardization, we used the first non-missing observation for each patient. We identified 101/510 (20%) positive for E484K, while 91/510 (18%) were positive for the N501Y variant. Four patients (0.8%) tested positive for both mutations. The mean (SD) Ct values were 21.6 (15.2−29.75), 21.7 (13.1−29.9) and 20.7 (13.0−29.5) for the E484K, N501Y and SARS-CoV-2 non-variant viruses, respectively. Ct Values ranged between 13 to 31.74 (Table 1). No significant difference in mean Ct values among the specimens positive for non-variant and variant viruses was observed (P-value = 0.16).

3.2. Correlation of RT-PCR mutation screen with WGS SARS-CoV-2 sequencing

In addition to validating the TaqMan assays performance characteristics, we assessed the assay analytical performance with WGS as a reference method. During that time, we tested 391 samples from unique patients by WGS and RT-qPCR. Among the samples assessed by both methods the sensitivity and specificity, for E484K and N501Y were 0.95 (95% CI, 0.880.99) and 1.00 (95% CI, 0.98−1.00) and 1.00 (0.94, 1.00) and 1.00 (0.99, 1.00), respectively (Table 2). Among the sequenced N501Y-positive samples we identified 61/391 (15.6%) positive by RT-qPCR. There were no false positive or false negative for N501Y by qRT-PCR (NPV and PPV, 100%, 95%CI, 0.99, 1.00). For E484K, there were 76 (19.4%) positive by both methods. Of the 80 samples positive by WGS 4 tested negative by RT-qPCR, while 1 sample was positive by RT-qPCR and negative by WGS. The positive and
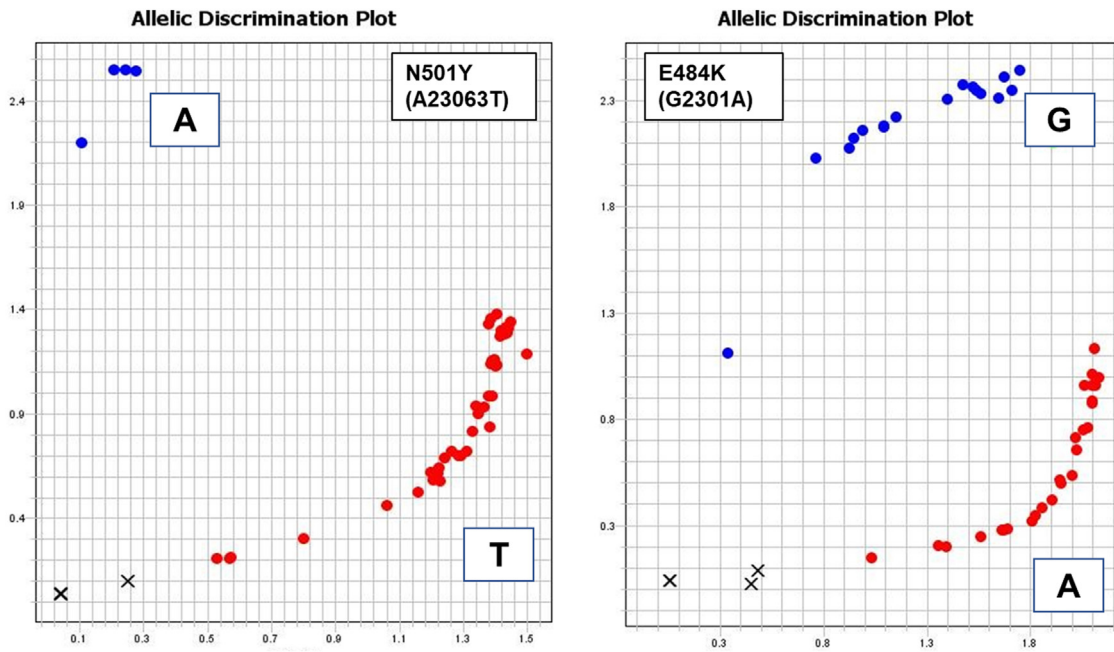

Fig. 1. Flowchart of samples assayed by TaqMan RT-qPCR and whole genome sequencing during the study period.

Fig. 2. Examples of TaqMan allelic discrimination plots. SARS-CoV-2 N501Y and E484K variants were amplified by sequence-specific primers followed by detection with 2 fluorogenic probes with different reporter dyes for each probe. Wild type (WT) variants are shown in red (VIC), while the mutated variants are shown in blue (6FAM). Sample position is determined by signal intensity of the corresponding variants on the X-axis and Y-axis. "X" indicates no template control (NTC) and failed amplification.

Table 1
Cohort demographics by SARS-CoV-2 variants.

Variable	Category	E484K: N = 94	N501Y: N = 86	Other: N = 349	P-value	Test
Gender	Female	44 (56 %)	34 (53 %)	173 (54 %)	0.91	χ2
	Male	34 (44 %)	30 (47 %)	148 (46 %)		
Age	Mean (SD)	44.65 (26.13)	44.78 (23.64)	49.07 (27.2)	0.22	Kruskal-Wallis
	-Median (IQR)	41.5 (25.25, 67.75)	46 (31.5, 61.25)	52 (29, 71)		
	-Range	(0.75, 96)	(0.17, 95)	(0.08, 101)		
Ct Value	Mean (SD)	21.59 (4.69)	21.71 (4.69)	20.68 (4.78)	0.16	Kruskal-Wallis
	-Median (IQR)	20.95 (18, 25.4)	21.2 (17.4, 26.6)	20.2 (16.8, 24.7)		
	-Range	(13.1, 29.9)	(15, 29.75)	(10.5, 29.8)		
Race/Ethnicity	Asian	12 (22 %)	5 (11 %)	44 (18 %)	0.35	χ2
	African American	12 (22 %)	8 (18 %)	34 (14 %)		
	Other	7 (13 %)	7 (16 %)	54 (22 %)		
	White	24 (44 %)	24 (55 %)	109 (45 %)		
	Hispanic or Latino	10 (19 %)	9 (22 %)	51 (22 %)	0.89	χ2
	No Hispanic or Latino	42 (81 %)	31 (78 %)	178 (78 %)		

Table 2
Validation of rapid RT-qPCR against whole-genome next-generation sequencing.

	Whole-genome sequencing results			
Mutation	E484K		N501Y	
RT-qPCR[a]	Detected	Not detected	Detected	Not detected
No. detected	76	1	61	0
No. not detected	4	310	0	330
Sensitivity	0.95 (0.88, 0.99)		1.00 (0.94, 1.00)	
Specificity		1.00 (0.98, 1.00)		1.00 (0.98, 1.00)
PPV (95% CI) [b]	0.99 (0.93, 1.00)		1.00 (0.99, 1.00)	
PPV (95% CI) [c]		0.99 (0.97, 1.00)		1.00 (0.99, 1.00)

[a] RT-qPCR relative to whole genome sequencing.
[b] PPV = positive predictive value.
[c] NPV = negative predictive value.

negative predictive values for E484K RT-qPCR were 99% (NPV, PPV, 95% CI, 0.93, 1.00 and 0.97, 1.00, respectively). The false positive sample had a Ct value of 15.1 and was classified as B.1.243. All the 4 false negative samples were classified as B.1.526. Of those only 1 had high Ct (29.44) that can explain the discrepancy. No repeat testing was performed; therefore, we cannot rule out pipetting or other errors.

The RT-qPCR successfully predicted the presence of B.1.1.7 and the 2 P.1/B.1351 VOCs, but it had significantly low sensitivity 62% (95% CI, 0.53, 0.71) for predicting B.1.526 variants (Table 3).

The distribution of PANGO lineage among the RT-qPCR screened samples is presented in Fig. 3, Table 4 and Suppl. Table 1. The earliest cases with B.1.1.7 and B.1.526 were identified in mid-January 2021,

Table 3
Comparison of whole-genome sequencing with the PCR mutation assays by lineage.

Mutation assay[a]	Whole-genome sequencing results					
	Detected	Not detected	Sensitivity	Specificity	PPV	NPV
B.1.1.7 (N501Y+)						
Detected	51	8	1.00 (0.93, 1.00)	0.98 (0.95, 0.99)	0.86 (0.75, 0.94)	1.00 (0.99, 1.00)
Not detected	0	328				
B.1.526 (E484K+)						
Detected	71	6	0.62 (0.53, 0.71)	0.98 (0.95, 0.99)	0.92 (0.84, 0.97)	0.86 (0.82, 0.90)
Not Detected	43	268				
P.1/B.1351 (E484K+/N501Y+)						
Detected	2	0	1.00 (0.16, 1.00)	-	-	-
Not Detected	0	0				
Wild-type (E484K-/N501Y-)						
Detected	212	0	0.95 (0.91, 0.98)	1.00 (0.97, 1.00)	1.00 (0.98, 1.00)	0.92 (0.86, 0.96)
Not Detected	11	127				

PPV = Positive predictive value; NPV = Negative predictive value.
[a] RT-qPCR relative to whole genome sequencing.

after which these variants were found in every batch of samples tested. The first cases of P.1 and B.1.351, harboring both p.N501Y and p.E484K were identified in mid-March 2021, with no substantial increase. Other cases of interest included the E484K positive R.1 and Q.3. By early March 2021, B.1.1.7 became the predominant strain in our cohort before surpassed by B.1.526. Among the 241 that were wild type (WT) by RT-qPCR, more commonly assigned lineages were B.1.2 (19%), B1 (15%), B1.1.434 (8%), B.243 (4%) and B.1575 (4%) (Fig. 2). Among 59 N501Y-positive specimens by RT-qPCR with lineage information, 53 (89%) were classified as B.1.1.7 and the remainder 11% were B.623 (5%) and B.1.604, B.1.1, B.1.2 each 2%. Among 77 samples with isolated E484K, 74 (96%) were classified as B.1.526 and

the remainder 4% were B.1.2, B.1.243, and B.1619. Two samples with E484K and N501Y sequencing information were P.1 and B.1.351 lineages. Of the 118 B.1.1526 variants by WGS 43 (36%) did not carry the E484K change, while all B.1.1.7 variants were N501Y-positive.

4. Discussion

The rapid emergence of SARS-CoV-2 VOC during 2020 necessitated the development of rapid diagnostics in support of clinical and surveillance needs. In this study we describe the development of mutation-specific RT-PCR assays to screen for SARS-CoV-2 variants rapidly and reliably in a clinical laboratory setting. These assays use
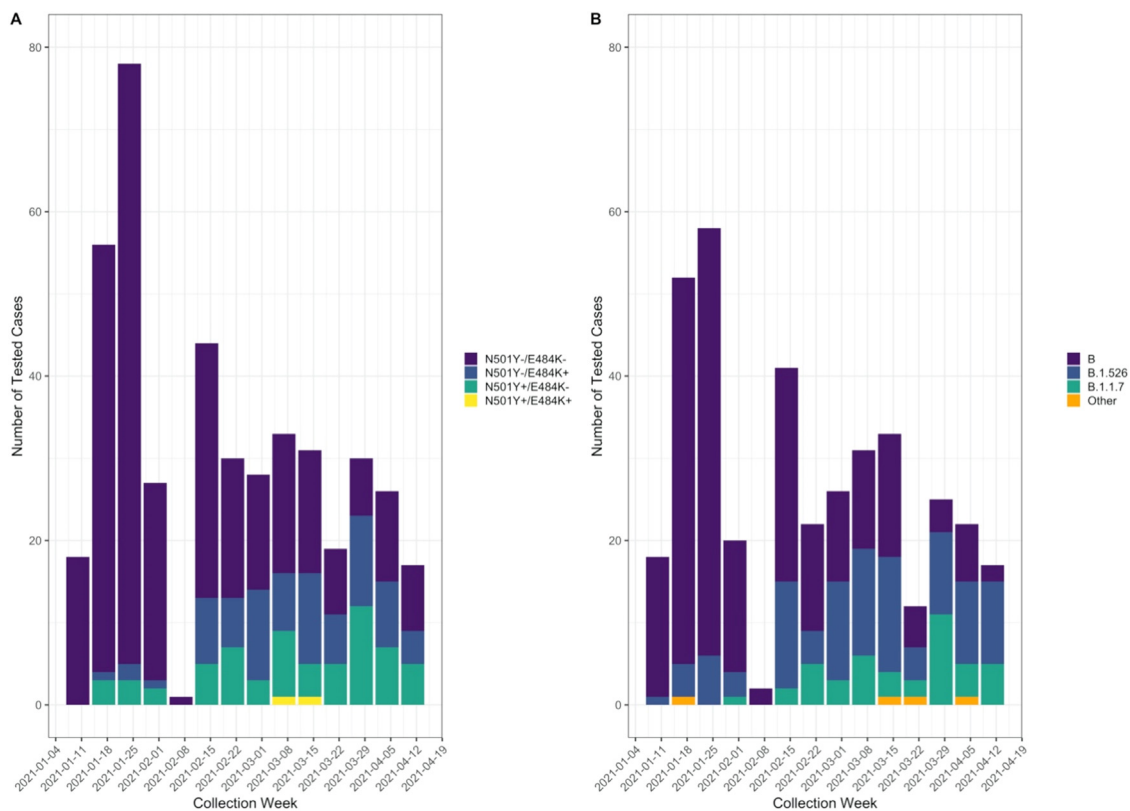

Fig. 3. Increasing prevalence of SARS-CoV-2 Spike E484K and N501Y variants from January to May 2021. The earliest detected variants in our study were collected in January. The number of cases per week for each combination of spike variants as detected by the rapid variant assay is shown in A and the number of cases per week for each pangolin lineage as determined from WGS is shown in B. Four cases of Other (non-B lineage) include C.31 (N501Y-/E484K-), C.37.1 (Lambda; N501Y-/E484K-), P.1 (Gamma; E484K+/N501Y+) and R.1 (N501Y-/E484K+) identified by WGS.

Table 4
SARS-CoV-2 lineage distribution of RT-qPCR screened variants.

PANGO lineage	RT-qPCR results			
	E484K (N = 73)	N501Y (N = 58)	N501Y and E484K (N = 2)	Other (N = 109)
B.1.1	0 (0 %)	1 (2 %)	0 (0 %)	4 (1 %)
B.1.1.7	0 (0 %)	50 (86 %)	0 (0 %)	0 (0 %)
B.1.2	1 (1 %)	1 (2 %)	0 (0 %)	51 (19 %)
B.1.243	1 (1 %)	0 (0 %)	0 (0 %)	10 (4 %)
B.1.351	0 (0 %)	0 (0 %)	1 (33 %)	0 (0 %)
B.1.411	0 (0 %)	1 (2 %)	0 (0 %)	0 (0 %)
B.1.526	69 (95 %)	0 (0 %)	0 (0 %)	44 (16 %)
B.1.604	0 (0 %)	1 (2 %)	0 (0 %)	0 (0 %)
B.1.619	1 (1 %)	0 (0 %)	0 (0 %)	0 (0 %)
B.1.623	0 (0 %)	3 (5 %)	0 (0 %)	0 (0 %)
P.1	0 (0 %)	0 (0 %)	1 (33 %)	0 (0 %)
Q.3	0 (0 %)	1 (2 %)	0 (0 %)	0 (0 %)
R.1	1 (1 %)	0 (0 %)	0 (0 %)	0 (0 %)

PANGO lineage classification performed with pangolin v2.4.1 and pangoLEARN June 5, 2021.

TaqMan chemistry to target 2 important sequence variants in the RBD spike region. The assay has excellent concordance with the SARS-CoV-2 variant mutations identified by WGS.

TaqMan-based real time PCR is an accurate, rapid, and a quantifiable method for detecting known single nucleotide variants (SNVs). The primers and probes design is flexible, allowing optimal placement of primers in the region flanking the SNV site and rapid implementation with newly emerging variants. The probe also includes a minor grove binder moiety greatly increasing the stability and specificity of the probe hybridization, and the use of an nonfluorescent quencher in place of the TAMRA quencher dye maximizes spectral performance.

SARS-CoV-2 has mutated innumerable times, with some variants potentially impacting efficacy of treatments and vaccines, stressing the importance of continued surveillance of viral changes. With the evolving virus, reliable and accurate information are critical for identifying these changes to be able make informed and effective decisions for overall public health. There have been 5 SARS-CoV-2 VOCs including the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta, and Omicron variants, as well as 4 variants of interest (VOIs) including the Eta, Iota, Kappa, and Lambda variants have been recognized by the WHO as of late November 2021. The spike p.N501Y and p.E484K have been identified as the most important functional changes common to all variants except the Delta, resulting in increased affinity of the spike protein to ACE2 receptors and subsequent enhanced entry into the host cells.

While WGS is required to confirm the identity of emerging variants, this technology is expensive and time consuming for routine use. Using TaqMan assays we were able to rapidly identify the emergence of B.1.1.7 and the B.1.526 variants in our cohort. The first cases were detected in early January 2021. By early spring these variants rose to alarmingly high numbers, accounting for approximately 70% of the cases in NYC. This agrees to other studies of these variants in NYC and elsewhere who reported similar findings [7,12]. The high percentage of the 2 variants highlights its apparent transmissibility compared to the WT variants that were concurrently circulating in NYC. Although, across states there has been an increase in B.1.1.7 as the growth of B.1.526 slowed down, indicative of the higher fitness of B.1.1.7 [13].

Mutation-specific assays for detecting SARS-CoV-2 mutations have now been implemented by other laboratories with great success [12,14–16]. In our study we found that while the N501Y mutation was an excellent marker for B.1.1.7, this was not the case for E484K and B.1.526. Although within B.1.526, the largest sub-clade is defined by E484K, 2 other distinct sub-clades are each defined by S477N; both mutations located within the RBD of spike. One of these sub-lineages defined by S477N has been later designated B.1.526.2. A closely related lineage, B.1.526.1 is defined by other spike mutations including L452R [13]. WGS studies have shown the early emergence of the B.1.526 E484-positive strain in multiple clonal backgrounds, including the B.1.243 that emerged in Arizona as well as B.1.1.7 +E484K in Pennsylvania [17–19]. Variants which contain the E484K mutations have been shown to be partially or completely resistant to certain therapeutic monoclonal antibodies, and less susceptible to neutralization by convalescent plasma shown to help fight SARS-CoV-2 infection [7,20], supporting the importance of accurate lineage determination. This approach also has excellent specificity and sensitivity in identifying VOC and related lineages such as the P.1 and B.1351 variants. Mutation specific assays are also superior to the use of S-gene drop out in the 3-target (H69-V70del) diagnostic RT-PCR assay reported by numerous labs for identifying B.1.1.7, since numerous non-VOC strains also carry the H69/V70 deletions [12,15,16]. Zelyas et al., in their study have identified numerous cases with the H69/V70 deletion without N501Y belonging to the B.1.525 lineage which was not considered a VOC [12].

In this study 5 N501Y and 3 E484K-positive samples were found to belong to other B.1 lineages that were not VOCs. This may be due to erroneous lineage assignments as well as other technical errors. All the B.1.1.7 variants were N501Y-positive. However, of the B.1.1526 positive by WGS 36% did not carry the E484K change. All these variants were also negative for the L452R mutation but were positive for S477N by WGS and thus designated as B.1.1526.2.

SARS-CoV-2 variants are characterized by their transmissibility, disease severity and ability to evade the immune system. The B.1.1.7 variant has been associated with increased transmissibility and potentially higher viral levels, while the B.1.526 E484K-positive variant (Iota) has shown resistance to therapeutic antibodies [3,7]. The present study did not identify any difference in age, gender, or race between the E484K or N501Y VOCs and the WT SARS-CoV-2 group (data not shown). There was also no difference in the samples Ct values between the different groups. Although N501Y was shown to have increased viral replication in human upper respiratory airways [3], other studies did not indicate a substantial difference in VOC transmissibility among different age groups [4].

One advantage of the TaqMan SNP genotyping assay used in this study is that it can be readily implemented in routine clinical work. It allows standardization and improved assay performance across laboratories compared to laboratory-developed tests. The assay is highly scalable, allowing testing a few or hundreds of samples to identify 1 or many mutations with a methodology that is well established. In our hands this enabled timely identification of the rise of cases with B.1.1.7 and B.1.1526 variants in our patient population. This is especially pertinent to rapid determination of appropriate therapy for individual patient care.

In summary, real time TaqMan assays is an effective method for rapid screening of known SARS-CoV-2 variants from patient specimen, applicable for most diagnostic laboratories within a pandemic setting. WGS is still required to confirm the identity of certain variants and for general surveillance.

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Declaration of competing interests

The authors report no conflicts of interest relevant to this article.

Authors' contributions

H.R. and P.V. designed the study, wrote the manuscript and and/or performed computational analyses. L.C., Z.Z., J.G, J.G., P.R., A.S. and K.F. planned or performed laboratory experiments. S.R., E.I., C.Z. and D.B. performed computational analyses and/or reviewed the manuscript. Y.Q. performed statistical analyses. L.W., L.M and M.C. reviewed the manuscript.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2022.115789.

References

[1] Lauring AS, Hodcroft EB. Genetic Variants of SARS-CoV-2-What Do They Mean? JAMA 2021;325(6):529–31.

[2] Callaway E. The coronavirus is mutating - does it matter? Nature 2020;585 (7824):174–7.

[3] Tao K, Tzou PL, Nouhin J, Gupta RK, de Oliveira T, Kosakovsky Pond SL, Fera D, Shafer RW. The biological and clinical significance of emerging SARS-CoV-2 variants. Nat Rev Genet 2021;22(12):757–73.

[4] Volz E, Mishra S, Chand M, Barrett JC, Johnson R, Geidelberg L, Hinsley WR, Laydon DJ, Dabrera G, O'Toole A, et al. Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England. Nature 2021;593(7858):266–9.

[5] Khare S, Gurry C, Freitas L, Schultz MB, Bach G, Diallo A, Akite N, Ho J, Lee RT, Yeo W, et al. GISAID's Role in Pandemic Response. China CDC Wkly 2021;3(49):1049–51.

[6] Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Munday JD, Pearson CAB, Russell TW, Tully DC, Washburne AD, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science 2021;372(6538).

[7] Annavajhala MK, Mohri H, Zucker JE, Sheng Z, Wang P, Gomez-Simmonds A, Ho DD, Uhlemann AC. A Novel SARS-CoV-2 Variant of Concern, B.1.526, Identified in New York. medRxiv 2021.

[8] Faria NR, Mellan TA, Whittaker C, Claro IM, Candido DDS, Mishra S, Crispim MAE, Sales FCS, Hawryluk I, McCrone JT, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. Science 2021;372(6544):815–21.

[9] Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, Doolabh D, Pillay S, San EJ, Msomi N, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. Nature 2021;592(7854):438–43.

[10] Velu P, Craney A, Ruggiero P, Sipley J, Cong L, Hissong EM, Loda M, Westblade LF, Cushing M, Rennert H. Rapid Implementation of Severe Acute Respiratory Syndrome Coronavirus 2 Emergency Use Authorization RT-PCR Testing and Experience at an Academic Medical Institution. J Mol Diagn 2021;23(2):149–58.

[11] Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv 2013.

[12] Zelyas N, Pabbaraju K, Croxen MA, Lynch T, Buss E, Murphy SA, Shokoples S, Wong A, Kanji JN, Tipples G. Precision Response to the Rise of the SARS-CoV-2 B.1.1.7 Variant of Concern by Combining Novel PCR Assays and Genome Sequencing for Rapid Variant Detection and Surveillance. Microbiol Spectr 2021;9(1): e0031521.

[13] West Jr AP, Wertheim JO, Wang JC, Vasylyeva TI, Havens JL, Chowdhury MA, Gonzalez E, Fang CE, Di Lonardo SS, Hughes S, et al. Detection and characterization of the SARS-CoV-2 lineage B.1.526 in New York. Nat Commun 2021;12(1):4886.

[14] Wang H, Miller JA, Verghese M, Sibai M, Solis D, Mfuh KO, Jiang B, Iwai N, Mar M, Huang C, et al. Multiplex SARS-CoV-2 Genotyping Reverse Transcriptase PCR for Population-Level Variant Screening and Epidemiologic Surveillance. J Clin Microbiol 2021;59(8):e0085921.

[15] Wang H, Jean S, Eltringham R, Madison J, Snyder P, Tu H, Jones DM, Leber AL. Mutation-Specific SARS-CoV-2 PCR Screen: Rapid and Accurate Detection of Variants of Concern and the Identification of a Newly Emerging Variant with Spike L452R Mutation. J Clin Microbiol 2021;59(8):e0092621.

[16] Lind A, Barlinn R, Landaas ET, Andresen LL, Jakobsen K, Fladeby C, Nilsen M, Bjornstad PM, Sundaram AYM, Ribarska T, et al. Rapid SARS-CoV-2 variant monitoring using PCR confirmed by whole genome sequencing in a high-volume diagnostic laboratory. J Clin Virol 2021;141:104906.

[17] Annavajhala MK, Mohri H, Wang P, Nair M, Zucker JE, Sheng Z, Gomez-Simmonds A, Kelley AL, Tagliavia M, Huang Y, et al. Emergence and expansion of SARS-CoV-2 B.1.526 after identification in New York. Nature 2021;597(7878):703–8.

[18] Moustafa AM, Bianco C, Denu L, Ahmed A, Neide B, Everett J, Reddy S, Rabut E, Deseignora J, Feldman MD, et al. Comparative Analysis of Emerging B.1.1.7 +E484K SARS-CoV-2 isolates from Pennsylvania. bioRxiv 2021.

[19] Skidmore PT, Kaelin EA, Holland LA, Maqsood R, Wu LI, Mellor NJ, Blain JM, Harris V, LaBaer J, Murugan V, et al. Genomic Sequencing of SARS-CoV-2 E484K Variant B.1.243.1, Arizona, USA. Emerg Infect Dis 2021;27(10):2718–20.

[20] Liu Z, VanBlargan LA, Bloyet LM, Rothlauf PW, Chen RE, Stumpf S, Zhao H, Errico JM, Theel ES, Liebeskind MJ, et al. Identification of SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization. Cell Host Microbe 2021;29(3):477–88 e4.