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Partial ORF1ab Gene Target Failure with Omicron BA.2.12.1

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ABSTRACT Mutations in the genome of SARS-CoV-2 can affect the performance of molecular diagnostic assays. In some cases, such as S-gene target failure, the impact can serve as a unique indicator of a particular SARS-CoV-2 variant and provide a method for rapid detection. Here, we describe partial ORF1ab gene target failure (pOGTF) on the cobas SARS-CoV-2 assays, defined by a ≥2-thermocycle delay in detection of the ORF1ab gene compared to that of the E-gene. We demonstrate that pOGTF is 98.6% sensitive and 99.9% specific for SARS-CoV-2 lineage BA.2.12.1, an emerging variant in the United States with spike L452Q and S704L mutations that may affect transmission, infectivity, and/or immune evasion. Increasing rates of pOGTF closely mirrored rates of BA.2.12.1 sequences uploaded to public databases, and, importantly, increasing local rates of pOGTF also mirrored increasing overall test positivity. Use of pOGTF as a proxy for BA.2.12.1 provides faster tracking of the variant than whole-genome sequencing and can benefit laboratories without sequencing capabilities.

KEYWORDS cycle threshold (CT) value, ORF1ab gene, partial ORF1ab gene target failure (pORF1ab), RT-PCR, SARS-CoV-2, whole-genome sequencing (WGS)

Mutations in the primer/probe binding sites of the SARS-CoV-2 genome can affect oligonucleotide binding and molecular test performance. Throughout the pandemic, a number of such mutations have been described, resulting in partial or complete PCR target failure (1). Mutations that impair diagnostic detection is a criterion that the United States (U.S.) Centers for Disease Control and Prevention (CDC) considers when classifying a novel SARS-CoV-2 lineage as a variant of concern (2). Most nucleic acid amplification tests (NAAT) that are used for *in vitro* diagnostic (IVD) clinical testing and have received emergency use

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authorization (EUA) from the U.S. Food and Drug Administration (FDA) employ multitarget assay design. This limits the diagnostic impact of a SARS-CoV-2 mutation that causes single-target failure, as the additional primer/probe target sequences remain unaltered and adequately detect the presence of viral nucleic acid.

Viral mutations can negatively affect assay performance, but these phenomena have proven useful in certain situations. Whole-genome sequencing (WGS) of SARS-CoV-2 remains inadequately implemented or unavailable in many medical centers across the United States due to logistical, cost, and time constraints. Moreover, full genomic characterization of circulating variants often takes multiple weeks from sample collection to data reporting to public health agencies. Specific mutations that yield unique NAAT performance characteristics can provide broader and more rapid assessment of circulating lineages than WGS. For example, the well-characterized 6-bp deletion in the spike gene resulting in the absence of amino acids H69/V70 (69 to 70del) results in complete loss of detection of the spike gene target on the Thermo Fisher Scientific TaqPath COVID-19 assay (Thermo Fisher Scientific, Waltham, MA), commonly termed S-gene target failure (SGTF). SGTF was a hallmark of the Alpha (B.1.1.7) variant allowing for sequence-free estimation of Alpha's emergence and prevalence in early 2021 (3). Utility in tracking SGTF was also observed in late 2021 with the introduction of the Omicron (B.1.1.529.1 [BA.1]) variant, which contained the same characteristic 69 to 70del. Rapid estimation of Omicron emergence was possible as the preceding dominant variant, Delta, did not harbor the 69 to 70del and was therefore S-gene target positive (SGTP) (4). Finally, in early 2022, SGTF tracking proved useful in monitoring the transition from BA.1 Omicron to BA.2 Omicron (B.1.1.529.2), as the latter lacked the 69 to 70del and was SGTP, which served as a reliable proxy for identifying BA.2 (5).

While the impact of the 69 to 70del was dramatic, resulting in complete SGTF, other genomic mutations may result in subtler changes, including abnormal variance between cycle threshold (C_T) values of multitarget SARS-CoV-2 assays (6–10). Here, we describe partial ORF1ab gene target failure (pOGTF) as detected by abnormal delta of the C_T values from the E and ORF1ab (dEO) targets of the cobas SARS-CoV-2 assay and cobas SARS-CoV-2 & influenza A/B assay (Roche Molecular Systems, Inc.; Branchburg, NJ) linked to BA.2.12.1, which has rapidly emerged in the U.S. in March and April 2022 and has become the dominant SARS-CoV-2 lineage in central New York state (11).

MATERIALS AND METHODS

Study population. Samples used in this study included upper respiratory specimens (e.g., nasopharyngeal, anterior nares, nasal swabs, and saliva) collected in a transport medium validated by the individual testing laboratory, obtained from patients undergoing standard of care testing for COVID-19 between 6 March 2022 and 16 April 2022, a period of time corresponding to MMWR epidemiologic weeks 10 to 15 (12). Samples included in the dEO analysis were tested for the presence of SARS-CoV-2 on the cobas SARS-CoV-2 or cobas SARS-CoV-2 & influenza A/B assays at the originating laboratory. Samples sent for SARS-CoV-2 WGS may have been tested on a variety of other FDA EUA SARS-CoV-2 diagnostic platforms, and not a cobas SARS-CoV-2 assay, depending on workflows and testing protocols of the originating laboratory. Laboratories contributing to this study and appropriate IRB determination include the Cleveland Clinic Foundation (CCF) Clinical Microbiology Laboratory (IRB no. 18-318), the Clinical Microbiology Laboratory of Columbia University Irving Medical Center (CUIMC; IRB no. AAT0123), the Clinical Microbiology and SARS-CoV-2 Molecular Testing Laboratories at the Hospital of the University of Pennsylvania (HUP; IRB no. 848605), the Clinical Microbiology Service at Memorial Sloan Kettering (MSK; IRB no. 18-491), the Clinical Microbiology Laboratory of Weill Cornell Medical Center (WCMC; IRB no. 20-03021671), the Barnes-Jewish Hospital Molecular Infectious Disease Laboratory and Washington University in St. Louis (WUSTL; IRB no. 20211131), and Clinical Virology Laboratory at Yale-New Haven Hospital (YNHH) and Yale School of Public Health (IRB no. 2000031374).

Cobas SARS-CoV-2 assays. The cobas SARS-CoV-2 and cobas SARS-CoV-2 & influenza A/B assays are available on the Roche cobas 6800 and 8800 analyzers (Roche Molecular Systems, Inc.). Both assays perform qualitative detection of SARS-CoV-2 using two genome targets: the ORF1ab gene that is specific to SARS-CoV-2 and the pan-sarbecovirus envelope (E) gene. Details on the oligonucleotide sequences and the specific genomic regions targeted by the NAAT are not publicly available. MS2 bacteriophage is used as an internal RNA processing control. RNA extraction, reverse transcription, target amplification, and result analysis all occur on the instrument. Positive results are determined by amplification curves that cross a predetermined threshold thus generating C_T values for these loci. These C_T values are available to the laboratory but are not included in patient reports by any of the performing laboratories. Only qualitative interpretations (SARS-CoV-2 detected, not detected, or presumptive positive) of the results are reported, which is congruent with the EUA instructions for use. Samples do not have to be positive for both gene targets to be called positive by the assay (ORF1ab gene-only detected, positive; E-gene-only detected, presumptive positive). However, only results positive for both targets were used

in this study to allow for C_T value comparison. No samples with E-gene C_T value of ≤30 and ORF1ab gene "not detected" were identified, and therefore requiring both targets to be positive did not exclude any samples. C_T values used in this study as well as dates of sample collection and/or testing were either directly obtained from instruments or extracted from the laboratory information system depending on the testing laboratory.

Identification of samples as dEO outliers. At all study sites, initial recognition of dEO was determined by manual review of C_T values, noticing ≥1 or ≥2 cycle difference between the ORF1ab and E C_T values, with ORF1ab C_T value greater than E-gene C_T value, depending on the institution. The exception was HUP, where a model of expected difference in C_T values between two parallel targets as a function of the minimum observed C_T value in the pair was used in order to accommodate the observation that the expected difference in C_T values across targets increases at higher C_T values. The expected variation in the C_T value difference was likewise allowed to vary with the minimum observed C_T value in the pair. Bayesian mixed-effect linear regression models, incorporating random effects (slope and intercept) for sequencing platform (i.e., amplicon-target pair), were fit using Stan Hamiltonian Monte Carlo via the brms package in R (13, 14). Model parameters were established using C_T value data collected across seven platforms from March to June 2021, and the parameterized model was used to monitor for dEO (and other target pair) outliers, with outliers identified as differences beyond the 99% posterior credible interval of the expected C_T value difference for the minimum C_T value observed in the pair. Model code and sample reproducible report are available at https://github.com/bjklab/SARS-CoV-2_Ct_report.

SARS-CoV-2 whole-genome sequencing. At CCF, NGS sequencing libraries were prepared with Illumina COVIDSeq test kit according to the manufacturer's recommendation. These libraries were sequenced paired end with read length of 151 bases using a NextSeq550 instrument (Illumina, San Diego, CA). Data analysis was performed with an in-house developed bioinformatics pipeline. Sequence reads were mapped to reference genome Wuhan-Hu-1 (NC_045512.2) using BWA (version 0.7.15), and variant calling was performed with both Freebayes (version 1.3.4) and LoFreq (version 2.1.5). Average coverage is ×6,567 and minimum coverage for mutation is ×10. Samples below this minimum coverage at the S-gene codon 452 were considered unreliable, and these genotypes were not considered in this study. Manual review of mutations was performed with Integrative Genomics Viewer (IGV) to remove any artifacts. Variant classification was performed with the Pangolin program (https://pangolin.cog-uk.io/ version v4.0.5, lineages version 2022-04-09). Derived genomes with related information were deposited into the GISAID database. Samples from CUIMC and WCMC campuses were sequenced using the Oxford Nanopore (Oxford Nanopore Technologies, Oxford, UK) Midnight protocol targeting 1,200-bp tiled amplicons across the length of the genome, as described previously (15). Samples were sequenced on an Oxford Nanopore GridION using R9.4.1 flow cells, with negative controls included on each run. Variant calling and consensus genome generation were performed using the Oxford Nextflow ARTIC pipeline. Viral lineage classification, identification of mutations, and phylogenetic analyses were performed using Pangolin v4.0.5 and Nextclade v1.11.0. Genomic data from CUIMC is routinely uploaded to GISAID and to GenBank (under NCBI BioProject PRJNA751551). At YNHH, HUP, and MSK, sequencing was performed as described previously, but Pangolin v4.0.5 was used (16–19). Sequencing results from WUSTL were not available at the time of writing.

Data analysis. All charts and analyses were generated and performed with GraphPad Prism v8.2.1. Nonlinear regression of BA.2.12.1 and non-BA.2.12.1 was performed, and best-fit straight lines using the least-squares method and *y* intercepts were compared for differences. Receiver operating characteristic (ROC) curve to determine the performance characteristics of different dEO cutoff values was generated by classifying samples with lineage information available as either "BA.2.12.1" or "Other." Positive and negative predictive values of pOGTF for the detection of BA.2.12.1 were calculated using samples with lineage information classified as either BA.2.12.1 or Other where pOGTF was considered a dEO of ≥2 with an E-gene C_T value of ≤30.

Data availability. All SARS-CoV-2 viral genomes have been deposited in GISAID and/or NCBI GenBank with GISAID accession numbers listed in Table S1 in the supplemental material.

RESULTS

Difference in C_T values for the E-gene target compared to those for the ORF1ab gene target (dEO) from 6 March 2022 to 16 April 2022 were plotted (Fig. 1, Table S1 in the supplemental material). Using an E-gene C_T value cutoff of ≤30 thermocycles to avoid nonspecific variation between values near the limit of detection of the assay, an outlier group with abnormal difference between the two C_T values was identified. These data indicate delayed detection of the ORF1ab target compared to that of the E-gene target. In total, 427 of 3,462 total positive samples, run on either the cobas SARS-CoV-2 assay and cobas SARS-CoV-2 & influenza A/B assay, exhibited pOGTF during the study period.

Following institution-specific workflows for SARS-CoV-2 surveillance sequencing, a subset of samples (832) from the study period underwent WGS and lineage determination, including 70 samples showing pOGTF (Table S1). Overwhelmingly, pOGTF samples were determined to be BA.2.12.1 (69/70), with the first WGS-confirmed detection of BA.2.12.1 among study participants occurring in the first week of March in the YNHH cohort and remaining study sites also confirming BA.2.12.1 from pOGTF specimens in late March. The single discrepant result, CCF-0259, demonstrated pOGTF but was identified as BA.2. The

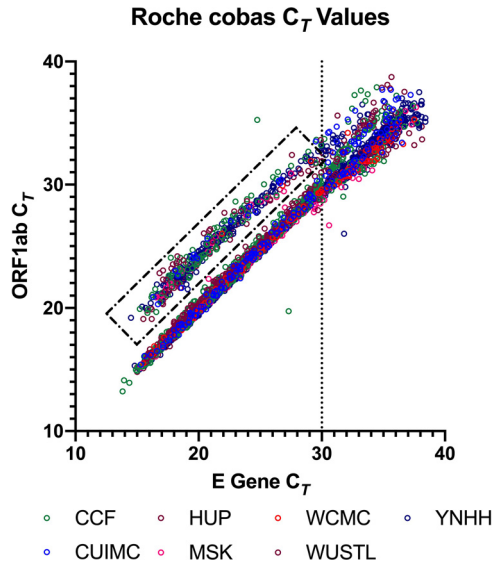

FIG 1 C_T values for ORF1ab and E-gene targets from cobas instruments. SARS-CoV-2 was detected in 3,462 unique samples from 6 March 2022 to 16 April 2022, and ORF1ab and E-gene C_T values are plotted. The results clustered into two visibly distinct groups. The upper-left group (dashed box) represents partial ORF1ab gene target failure (pOGTF), and the lower-right group has no target failure. The dashed vertical line indicates an E-gene C_T value of 30 that was used as a cutoff for subsequent analyses. Samples with only a single gene detected were not included and are not shown. Abbreviations: Cleveland Clinic Foundation (CCF), Columbia University Irving Medical Center (CUIMC), Hospital of the University of Pennsylvania (HUP), Memorial Sloan Kettering (MSK), Weill Cornell Medical Center (WCMC), Washington University in St. Louis (WUSTL), and Yale-New Haven Hospital (YNHH).

remaining 762 non-pOGTF samples sequenced comprised various other lineages (Table S1), with only one determined to be BA.2.12.1 (YNHH-0835). The emergence of samples with pOGTF coincided with increases in BA.2.12.1, and preliminary evidence suggested that pOGTF may be a marker for BA.2.12.1. To explore this, ORF1ab and E-gene C_T values were plotted for samples confirmed as BA.2.12.1 or another lineage by sequencing. BA.2.12.1 samples also clustered as dEO outliers (Fig. 2). Best-fit straight lines demonstrated these groups to be different populations with significantly different *y* intercepts ($P < 0.0001$). Further analysis of sequencing data demonstrated that BA.2.12.1, a sublineage of BA.2, contains at least five characteristic mutations of interest to this study compared to the parent strain. These include two missense mutations altering amino acids in the spike gene, g.22917 T > G (S:L452Q) and g.23673 C > T (S:S704L), and three synonymous mutations, g.11674 C > T (ORF1ab), g.15009 T > C (ORF1ab), and g.21721 C > T (S). Likely, one of the two ORF1ab synonymous mutations causes reduced efficiency of amplification and/or detection of the target in the cobas SARS-CoV-2 and cobas SARS-CoV-2 & influenza A/B assays, which results in pOGTF and abnormal dEO.

We used the subset of samples with lineage information to generate an ROC curve to determine the performance characteristics of different cutoff values for dEO (Fig. 3). At a dEO cutoff of 2.1, the sensitivity was 98.6% while the specificity was 99.9% generated during the ROC analysis. After review of the primary data, there was no difference in performance using a cutoff of 2.0 versus 2.1, and thus dEO of ≥2.0 was selected for simplicity of use in identifying samples with pOGTF (Table 1 and 2). When this criterion was applied to our population of samples with lineage information with E-gene C_T values of ≤30, the positive and negative predictive values were 98.6% and 99.9%, respectively. Using the thresholds of E-gene C_T values of ≤30 and dEO of ≥2 to define pOGTF, we reanalyzed the cobas SARS-CoV-2 and cobas SARS-CoV-2 & influenza A/B results from all study sites, finding that the pOGTF group showed a mean dEO of 4.02 (95% confidence interval [CI] of 3.95 to 4.09) cycles compared to −0.13 (95% CI of −0.15 to −0.11) cycles in the non-pOGTF group (Table 3).

To better understand the relationship between pOGTF, the emergence of BA.2.12.1, and overall test positivity, we reviewed publicly available GISAID data to identify the

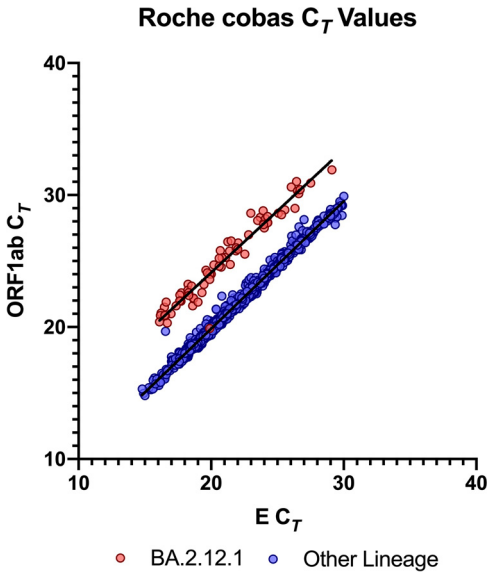

FIG 2 SARS-CoV-2 lineage designation and cobas C_T values. SARS-CoV-2 lineage was determined for 832 samples, including 70 samples with BA.2.12.1 (red) and 762 samples with other lineages (blue). ORF1ab and E-gene C_T values are plotted, and the BA.2.12.1 group demonstrates partial ORF1ab gene target failure (pOGTF) as indicated by the elevated *y* intercept. Linear regression was performed, and best-fit curves for BA.2.12.1 and all other lineages had significantly different *y* intercepts ($P < 0.0001$). Only samples with E-gene C_T values of ≤30 were used for regression analysis.

number of all sequences and BA.2.12.1 sequences uploaded from North America for the same time period as our study. Over a 6-week period, the number of sequences submitted was stable, but the proportion of BA.2.12.1 samples increased over time to approximately 17% of all sequences (Fig. 4A). Our total testing data using the pOGTF threshold also revealed a similar rise in samples demonstrating pOGTF, with the proportion of positive

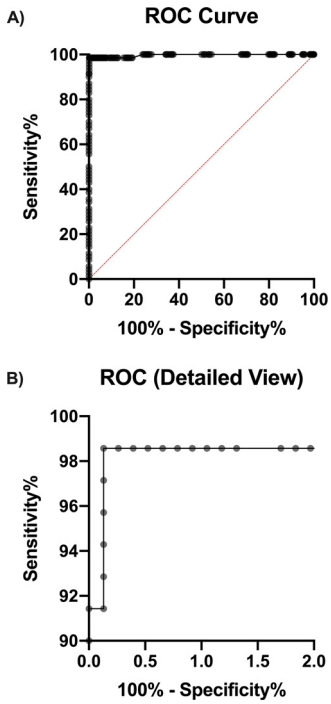

FIG 3 ROC curve analysis for dEO as a marker for BA.2.12.1. For samples with available sequencing data, dEO was calculated, and samples were classified as "BA.2.12.1" or "Not BA.2.12.1." A total of 70 BA.2.12.1 and 762 other lineage samples were included. ROC analysis was performed in GraphPad Prism, and full ROC curve (A) and a detailed view (B) are shown. Only samples with E-gene C_T values of ≤30 were used.

TABLE 1 Contingency table using dEO threshold of ≥2 for samples with E-gene C_T of ≤30 as a marker of BA.2.12.1

Data analyzed	BA.2.12.1 = yes	BA.2.12.1 = no	Total
pOGTF = yes	69	1	70
pOGTF = no	1	761	762
Total	70	762	832

samples with pOGTF steadily increasing over the study period, peaking in the final week of the study (April 10 to 16) (Fig. 4B). This rise is continuing. Importantly, the rise in pOGTF at our institutions has also corresponded to an increase in overall test positivity, consistent with concerns of increased transmissibility of the BA.2.12.1 variant.

DISCUSSION

Roles for C_T values have been hotly debated throughout the pandemic (20–30). Multiple sources of preanalytic and analytic variability, along with the qualitative nature of SARS-CoV-2 assays, can complicate interpretation, particularly on a patient-by-patient basis (31–33). We find the diagnostic implication of target failure, both full or partial, to be useful. With enough data points, a normal dispersion between C_T values of multitarget assays can be constructed and monitored for evidence of abnormal differences in C_T values between two targets (Fig. 1) (6, 7). Abnormal divergence between C_T values may indicate polymorphisms that affect detection of individual target genes, particularly when the values are well above the limit of detection (6–10). Outliers showing abnormally large differences between targets or delayed detection of a given target relative to that of other targets may indicate mutations affecting the primer/probe binding sites and warrant further characterization by WGS. Unfortunately, target failure cannot always be assessed by the user. Target failure can potentially be assessed if the following are true: (i) RT-PCR is used, (ii) C_T values are visible to the user, (iii) each target being probed uses a discrete fluorophore, and (iv) a target is detected within the linear dynamic range of the assay.

Tracking unique assay performance characteristics as a marker of a specific variant has demonstrated value through more rapid assessment than WGS, as has been readily apparent with SGTF in the Alpha and Omicron (BA.1 and BA.2) waves of the COVID-19 pandemic. Here, we describe the use of pOGTF on the cobas SARS-CoV-2 and cobas SARS-CoV-2 & influenza A/B assays to rapidly identify the emergence of BA.2.12.1. pOGTF is a highly reliable proxy for BA.2.12.1, particularly for samples with C_T values of ≤30 thermocycles. This phenomenon is useful for rapid recognition, particularly for laboratories that lack sequencing capabilities. The CDC Nowcast variant proportion projection for the week ending 30 April 2022 estimates BA.2.12.1 at 36.5% (28.9 to 44.9%) nationally, with higher proportions in the Northeast of the United States (34). Our data are congruent with these projections, showing highest concentration in the U.S. Northeast with spread into the U.S. Midwest through mid-April, and we demonstrate that pOGTF can be used for real-time BA.2.12.1 tracking. Notably, estimates of the BA.2.12.1 variant fraction using pOGTF are higher than WGS-derived projections.

TABLE 2 Performance analysis using dEO threshold of ≥2 for samples with E-gene C_T of ≤30 as a marker of BA.2.12.1[a]

Affected characteristic	Value	95% CI
Sensitivity	0.9857	0.9234 to 0.9993
Specificity	0.9987	0.9926 to 0.9999
Positive predictive value	0.9857	0.9234 to 0.9993
Negative predictive value	0.9987	0.9926 to 0.9999
Likelihood ratio	751.1	

[a]pOGTF for BA.2.12.1 was detected using two-sided Fisher's exact test with a *P* value of <0.0001, indicating statistical significance ($P < 0.05$).

TABLE 3 Descriptive statistics for the entire data set for samples with E-gene C_T of ≤30

Characteristic	pOGTF = yes (dEO ≥2)	pOGTF = no (dEO <2)
No. of values	427	2,208
Minimum	2.200	−7.570
25% percentile	3.600	−0.3000
Median	4.000	−0.2000
75% percentile	4.400	0.05000
Maximum	10.50	1.900
Range	8.300	9.470
95% CI of median		
Actual confidence level	95.80%	95.00%
Lower confidence limit	3.900	−0.2000
Upper confidence limit	4.040	−0.2000
Mean	4.021	−0.1301
Std. deviation	0.7207	0.3699
Std. error of mean	0.03488	0.007872
Lower 95% CI of mean	3.952	−0.1455
Upper 95% CI of mean	4.089	−0.1146
Skewness	1.815	−3.163
Kurtosis	14.95	74.78

This increased fraction may indicate that BA.2.12.1 is spreading more quickly than has been estimated.

Two pOGTF discrepancies were identified: a BA.2 lacking the BA.2.12.1-related ORF1ab mutations, yet exhibiting pOGTF, and a BA.2.12.1 with all expected ORF1ab polymorphisms present but lacking pOGTF. The reason for these discrepancies is unknown but may include many sources of error from preanalytical factors through sequence data analysis.

The emergence of BA.2.12.1 at our study sites has coincided with increased positivity rates (Fig. 4), suggesting that this variant is at least partially responsible for increased community transmission. Increased transmissibility is likely attributable in part to the spike L452Q mutation present in BA.2.12.1, which is not found in the parent strain but was present in Lambda (C.37). A similar mutation, L452R, was observed with Delta (B.1.617.2) and is present in BA.4 (B.1.1.529.4) and BA.5 (B.1.1.529.5). L452Q has been implicated as an important driver of human transmission, enhancing infectivity and receptor binding while reducing vaccine-derived immunity (35).

For routine diagnostic laboratories, identifying mutations responsible for altering commercial assay performance is hindered by a lack of public information regarding the primer/probe target regions of commercially available FDA EUA assays. As stated during the 26 July 2021 CDC Clinical Laboratory COVID-19 Response Call, ". . .ideally genomic regions targeted by IVDs would be made publicly available by manufacturers, so prospective investigation of polymorphisms occurring within target regions could be identified" (36). In the context of a global pandemic and under FDA EUA, the authors support disclosure of primer/probe target regions to facilitate monitoring for mutations that could affect diagnostic assay performance. We acknowledge the potential financial risks in publishing propriety sequences but feel that this is appropriate during a Declaration of a Public Health Emergency. As in the case of pOGTF with BA.2.12.1, two putative mutations in ORF1ab (g.11674 C > T and g.15009 T > C) have been identified, but the responsible mutation affecting the assay performance has not yet been confirmed. If the former, this supports Wang et al.'s conclusion that cytidines in assay target regions are particularly susceptible to mutations for SARS-CoV-2 (37). Additional challenges for sentinel laboratories in identifying assay-affecting mutations are the frequency of the observation and quantity of testing. Sentinel laboratories may struggle to identify an emerging pattern when restricted to local data. It may therefore be easier to identify target failure using larger data sets or by retrospectively retesting

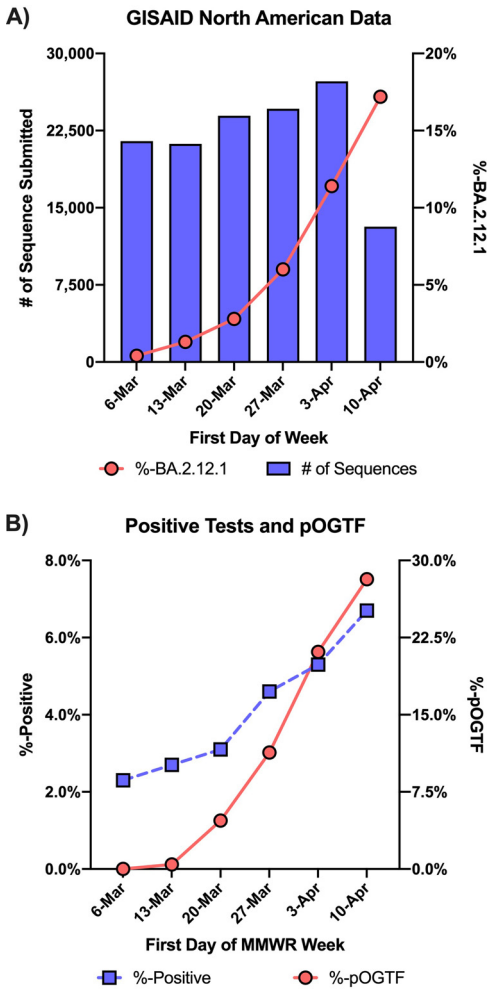

FIG 4 Increasing BA.2.12.1 prevalence relates to pOGTF and local positivity. (A) All North American samples submitted to GISAID from the indicated period of time were extracted on 2 May 2022, and the percentage of all sequences classified as BA.2.12.1 was calculated. (B) Percent positivity and percent partial ORF1ab gene target failure (pOGTF) for all samples tested by cobas SARS-CoV-2 or cobas SARS-CoV-2 & influenza A/B assays at participating institutions for the indicated weeks. For Cleveland Clinic data, percent positive data included additional samples tested by an alternative platform. All samples regardless of E-gene C_T value were included. Percent pOGTF was calculated for all samples with E-gene C_T values of $\leq$30 with or without a delta of the C_T values from the E and ORF1ab (dEO) of $\geq$2 cycles.

samples with mutations identified by WGS if the genomic regions targeted by the NAAT are publicly available.

Currently, mutations affecting primer/probe binding are self-monitored by manufacturers, which may pose a conflict of interest (38). Recognition of a mutation negatively affecting test performance could require assay redesign, revalidation, and resubmission to FDA for updated EUA, posing a number of potential commercial risks. Monitoring relies on publicly generated and openly shared genomic data (GISAID and GenBank), but the lag in availability of WGS data may delay detection of new variants that affect assay performance by weeks. Additionally, if a variant emerges in a locale with low sequencing surveillance, recognition may be delayed further. As demonstrated herein, monitoring of assay performance through real-time evaluation of C_T values has utility in SARS-CoV-2 variant surveillance and could inform clinical and logistical decision making. Widespread real-time uploading of SARS-CoV-2 NAAT C_T values to an FDA or manufacturer-sponsored database, similar

to the data curation available for BioFire Syndromic Trends Epidemiology Tool, could permit rapid detection, and thus response, to emerging variants (39).

This study has some limitations. Not all SARS-CoV-2 tests were performed on the cobas SARS-CoV-2 assays as multiple NAAT platforms are used at each participating site, and operational workflow may have unintentionally introduced biases (e.g., directing samples from certain patient populations or need for rapid result to particular platforms) to the study set. All analyses were performed on a per-sample and not a per-subject basis, and a single subject's samples could have been included multiple times within the data set. However, the impact of this possibility is diminished by the multicenter nature of this study and large amount of data. While pOGTF is currently specific to BA.2.12.1, the specificity, sensitivity, and predictive values may change over time as SARS-CoV-2 continues to evolve and the lineages prevalent in the population changes. Additionally, the same data set used to generate the dEO cutoff was used to establish these performance characteristics. Although pOGTF can be a rapid and useful tool for monitoring BA.2.12.1, WGS remains important in confirming the lineage, assessing for additional mutations, and detecting new variants that decrease the specificity of the association of pOGTF with BA.2.12.1. To our current knowledge, the pOGTF phenomenon is limited to the cobas assays performed using the 6800 or 8800 test systems, and the findings of this study are not generalizable to other test systems.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 1.1 MB.

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