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Opinion

Laboratory Action Plan for Emerging SARS-CoV-2 Variants

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Background

In December 2020, a clinically significant SARS-CoV-2 variant with an increased transmission rate was identified in the United Kingdom (1–3). Currently referred to as SARS-CoV-2 VOC-202012/01 (Variant of Concern, year 2020, month 12, variant; VOC-202012/01) or the B.1.1.7 variant, it has been identified in multiple countries throughout the world and continues to spread unabatedly (1, 4). VOC-202012/01 appears to be detectable by commonly used nucleic acid-based tests with emergency use authorization (EUA) from the United States Food and Drug Administration (FDA), and hopefully will not present a diagnostic challenge for clinical laboratories. As the COVID-19 pandemic continues, we anticipate additional clinically significant SARS-CoV-2 variants will emerge and we propose several preparatory actions for clinical laboratories.

VOC-202012/01 was identified in a genomic sequencing investigation of a rapid increase in COVID-19 cases in South East England. VOC-202012/01 was detected in approximately 50% of the increased cases. A retrospective investigation identified the earliest case from September 20, and by December 13 there were 1108 cases identified (1, 2, 5). Despite increased potential transmissibility, there is currently no evidence indicating increased severity of disease or decreased efficacy of vaccines of this variant (4). Reassuringly, antibody responses to vaccine epitopes indicate the genetic changes of the new variant are outside of major antibody binding sites (6). An evaluation of the Pfizer/BioNTech vaccine suggests efficacy against VOC-202012/01 (7).

Genetic Changes in VOC-202012/01

VOC-202012/01 has 17 genetic changes affecting amino acid sequence (missense, deletions, early stop codon) (2) (Table 1); 8 of these genetic changes occur in the S (spike) gene domain. The overall number of genetic changes is considered large compared to other SARS-CoV-2 variants. Globally, most SARS-CoV-2 variants have only a few genetic changes and are estimated to accumulate at a rate of 1–2 per month (2, 8). Current speculation is that the high number of genetic changes in VOC-202012/01 may be due to a rapid selection pressure event such as a prolonged infection in a patient with reduced immunocompetence (1, 9, 10) or that the variant developed within an infected animal and was transmitted back to humans (1, 11). However, in general, coronavirus genomes are relatively genetically stable, particularly when compared to RNA viruses such as influenza A virus. This is because coronaviruses encode a proof-reading mechanism to reduce mutations, whereas influenza A virus and many other RNA viruses have low fidelity, error-prone polymerases (12, 13). Thus, additional knowledge about host pressures that led to the emergence of VOC-202012/01 will be informative from the standpoint of viral evolution.

Laboratory Impact

VOC-202012/01 genetic changes are present in multiple genes, including those commonly targeted by SARS-CoV-2 diagnostic tests: ORF1ab, S-gene, N-gene. As of January 8, 2021, there are 2 nucleic acid-based tests known to be impacted by VOC-202012/01 [TaqPath COVID-19 Combo Kit (TaqPath), Linea COVID-19 Assay Kit (Linea)]; a third test [MesaBiotech Accula (Accula)], may be affected by SARS-CoV-2 variants with a genetic change not present in VOC-202012/01 (14). The TaqPath and Linea assays are impacted by S-gene genetic changes in VOC-202012/01; however, the overall sensitivity of these 2 assays is not known to be affected (14). The TaqPath assay is a multiplex real-time reverse transcription PCR test that targets the ORF1ab, S and N genes; only the S-gene target is affected due to deletion of genome nucleotides 21765_21770del (His69_Val70del). The Linea assay includes 2 targets within the S-gene; only one target is affected. A patient sample with VOC-202012/01 will have a negative amplification signal in the S-gene target, but positive amplification signals in the ORF1ab and N-gene targets using the TaqPath assay (2 of 3 targets positive) and the Linea assay will yield 1 of 2 positive S-gene targets. Of note, the implicated deletion (His69_Val70del) is

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Table 1. Nucleotide variation in SARS-CoV-2 VOC 202012/01 (2).

Gene	Nucleotide change	Amino acid[a]	Notes
ORF1ab	3267C>U	Thr1001Ile	
	5388C>A	Ala1708Asp	
	6954U>C	Ile2230Thr	
	11288_11296del	Ser3675_Phe3677del	
S-gene (spike)	21765_21770del	His69_Val70del	Negative S-gene in TaqPath
	21991_21993del	Tyr144del	
	23063A>U	Asn501Tyr	Amino acid in the receptor binding domain
	23271C>A	Ala570Asp	
	23604C>A	Pro681His	
	23709C>U	Thr716Ile	
	24506U>G	Ser982Ala	
	24914G>C	Asp1118His	
ORF8	27972C>U	Gln27*	
	28048G>U	Arg52Ile	
	28111A>G	Tyr73Cy	
N-gene (nucleocapsid)	28280_28282delinsCUA	Asp3Leu	
	28977C>U	Ser235Phe	

[a] UCSC Genome Browser on SARS-CoV-2 Jan. 2020/NC_045512.2 Assembly (wuhCor1);
*, stop codon.

not exclusive to VOC-202012/01. Manufacturers of other major assays have thus far reassured laboratories that there is no predicted impact on individual target detection or qualitative detection of SARS-CoV-2 due to the genetic changes in VOC-202012/01 or other SARS-CoV-2 variants.

Since the emergence of SARS-CoV-2, clinical laboratories, regulators, and manufacturers have anticipated that genetic changes could occur and potentially impact testing. At the outset of the pandemic, since there was limited knowledge of the conserved genetic regions of SARS-CoV-2, diagnostic assays were made with multiple targets to enable positive detection even if a single target became impacted by genetic changes. However, single-target SARS-CoV-2 nucleic acid tests were also developed due to early challenges in validating and maintaining adequate supplies; these single-target assays remain in use. As of January 13, 2021, 83% (152 of 192) of nucleic acid amplification assays with FDA EUA have more than one target for detection (15).

Action Plan

Clinical laboratories should develop a strategy for how they will address VOC-202012/01 and prepare for new SARS-CoV-2 variants. As the pandemic continues, there is a possibility of future genetic changes that significantly impact test performance.

1. ASSESS THE IMPACT OF NEW VARIANTS ON VIRAL DETECTION WITH EXISTING PLATFORMS

To date, changes in detection of VOC-202012/01 are not reported for any assays with US FDA EUA and the only individual target with reported altered detection is the S-gene within the TaqPath and Linea assays; other S-gene-targeting assays are not apparently affected (14).

Assurances of assay performance by manufacturers have been mostly based on in silico analyses. When the designs of primers/probes are publicly available, laboratories should perform in silico analysis to assess the potential impact. In addition, in silico predictions should be confirmed on biologic materials (e.g., patient samples or inactivated virus). Genetic changes may not cause a total loss in signal, but may lead to decreased (or increased) detection efficiency. Therefore, laboratories should consider confirming assay limit of detection when: (*a*) the new variant becomes prevalent within the population tested, and (*b*) quantified reference materials become available to determine analytic sensitivity.

Laboratories should work with manufacturers, regulators, and public health authorities to quickly coordinate an approach to identifying and distributing emerging SARS-CoV-2 variants to challenge existing assay systems. At a minimum, assays with EUA should be assessed for the potential impact of SARS-CoV-2 variants by direct laboratory examination in addition to in silico predictions. While the current focus is on nucleic acid tests, the impact on antigen tests warrants further investigation.

2. DEVELOP APPROACHES FOR VARIANT SCREENING

After recognition of an emerging variant, screening protocols need to be developed. For example, the TaqPath assay has been implemented as part of a two-step screening process for VOC-202012/01 where positive samples with a negative S-gene target are further evaluated by whole-genome sequencing (16). This strategy effectively identifies the His69_Val70del but is not specific for VOC-202012/01. Public Health England used this two-step strategy in their investigation of VOC-202012/01 (5). In the US, a two-step process screening with S-gene negative tests (e.g., TaqPath) reflexing to whole-genome sequencing is feasible, but coordination and centralization of data need to be implemented. There are multiple laboratories in the US that provide SARS-CoV-2 whole-genome sequencing for diagnosis; these laboratories need to be coordinated with existing genomic sequencing surveillance networks. The integration of testing laboratories, public health authorities, and genomic surveillance networks needs to occur for the rapid identification of not only VOC-202012/01 but additional emerging SARS-CoV-2 variants.

3. ACTIVELY MONITOR FOR EMERGENCE OF VARIANTS IN THE CLINICAL LABORATORY

While most clinical laboratories are not equipped to perform extensive surveillance, many assays provide information that can be leveraged as first-line surveillance tools. For example, laboratories performing multitarget assays that report individual target cycle threshold (C_t) values should consider monitoring the expected relative differences in C_t values of multiple targets. Patient samples with discordant results across multiple target genes should be investigated (e.g., negative S-gene in an overall positive TaqPath assay). If multiple samples have the same pattern of target gene discordance, especially when high viral loads are detected, these data should be shared with public health authorities and further investigated. Whenever feasible, laboratories should correlate epidemiologic data with the emergence of samples with target gene discordance. The collection date and origin should be monitored, and public health laboratories should consider associating the emergence of variants with illness severity or clusters within household or contacts.

Additionally, laboratories routinely employing genome sequencing-based assays, such as the Illumina COVIDSeq Test or BillionToOne qSanger-COVID-19 Assay, may consider performing variant monitoring as part of their routine quality assessment program.

4. CONSIDER ENHANCED RETROSPECTIVE TESTING FOR SPECIAL POPULATIONS

Chronic infection can occur in immunocompromised hosts resulting in long-term infectious viral shedding and constant turnover of viral variants up to 100 days after infection (17). To better understand the evolution of SARS-CoV-2 and monitor for emerging or highly divergent variants, there should be consideration for specialized testing of patients with prolonged COVID-19 positivity (e.g., multiple strong positive nucleic acid test results over at least a month). Furthermore, patients with prior documented COVID-19 that have a new positive SARS-CoV-2 test results after a period of negativity (reinfection), or >90 days after their first documented positive should be considered for more intensive evaluation of novel variants (e.g., whole-genome sequencing).

Conclusions

VOC-202012/01 is a strong reminder that virus evolution and genetic variation can impact both their fitness and their detection by diagnostic assays. Although laboratories throughout the world have already weathered a year of relentless challenges in the COVID-19 pandemic, there is much more work to be continued. Addressing VOC-202012/01 and other emerging SARS-CoV-2 variants is the first task of the new year.

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