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CORRESPONDENCE

Modifying a Diagnostic SARS-CoV-2 Spike PCR to Turn a Del69/70 Dropout into a Discriminatory On-Target Assay

To the Editor-in-Chief:

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With great interest, we read the recent article by Zhen and Berry[1] presenting a novel first-line diagnostic quantitative PCR assay for SARS-CoV-2 detection, targeting the viral S gene. Following reports of increasing abundance of SARS-CoV-2 variants, such as VOC202012/01 in Britain and 501.V2 in South Africa, a number of single nucleotide polymorphisms and in-frame deletions within the S gene have come into the spotlight in recent weeks.[2] These mutations are increasingly attracting attention due to their potential consequences for infection control and vaccination programs.

Notably, Spike-del-HV69/70 (in-frame deletion) is present in 6.82% of all sequences listed in the GISAID database (351,996 entries as of January 17, 2021), including, for example, the B.1.1.7 lineage (VOC202012/01) and the Mink cluster V lineage.[2] Presence of the del-HV69/70 mutation leads to a complete loss of signal with Zhen and Berry's quantitative PCR assay[3] due to the localization of its TaqMan probe's (Thermo Fisher Scientific, Waltham, MA) target sequence (21,759 to 21,782) spanning across the mutated region. As a result, variants like B.1.1.7 would be systematically missed by the assay. This, however, creates the opportunity to create a second TaqMan probe based on Zhen and Berry's design to target del-HV69/70 variants specifically, for example, using locked nucleic acid bases to increase melting temperature (Probe 2, 5′YakimaYellow-TGG TCC CAG (+A)(+G)A T(+A)G C(+A)T-BHQ1).

We have successfully used this modified version of the assay as part of typing panels to screen for SARS-CoV-2 variants. A protocol and clinical validation panel for a manual single-plex application can be found on the protocols.io website (*https://www.protocols.io*; last accessed April 29, 2021) Furthermore, we have integrated a modified version of the assay into a fully automated high-throughput multiplex application for a diagnostic multitarget SARS-CoV-2 PCR test with integrated typing functionality, taking advantage of its high analytic sensitivity in addition to the ability to differentiate for the deletion.[3]

Including the additional TaqMan probe (Probe 2) in Zhen and Berry's assay is a simple way to restore inclusivity of the test for del-HV69/70 variants, without making any further changes to the original primer/probe set. As an additional benefit, the dual-probe approach allows the rapid discrimination between del-HV69/70 variants and HV69/70-WT, for example, to screen for B.1.1.7 prior to sequencing. It should be noted that the detection of del-HV69/70 is not sufficient to confirm a B.1.1.7 lineage isolate. Further tests for N501Y and P681H, for example, can increase confidence in the call, and next-generation sequencing should be considered the definite result.

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Ethical approval: Not applicable.

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Authors' Reply

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The emergence of multiple mutations in the SARS-CoV-2 viral genome have attracted global attention because of the following main concerns: i) SARS-CoV-2 variants of concern (VOC) that have the potential to cause enhanced transmissibility and/or increased disease severity; ii) failure of diagnostic assays due to mutations in the target gene sequences, resulting in decreased sensitivity and false-negative test results; and iii) variants negatively impacting the current vaccines and therapeutic treatments, such as monoclonal antibodies, rendering them less effective.[1–3] Currently, whole genome sequencing–based methodologies play a pivotal role in surveillance and identification of SARS-CoV-2 VOC across the globe. However, there is an urgent need to develop rapid and largescale testing strategies for the identification of SARS-CoV-2 VOC to enhance surveillance.

We thank Nörz et al for incorporating our work into their rapid and cost-effective assay designed for the identification of SARS-CoV-2 VOC containing the HV69/70 deletion and N501Y mutations. As different approaches for the identification of potentially clinically relevant variants emerge, the approach taken by Nörz et al is clearly one way to expedite the design and the implementation of testing variants in many clinical laboratories through the utilization of existing equipment and expertise. In addition, the use of previously published, peer-reviewed assays that can not only identify the wild-type version of SARS-CoV-2 with high sensitivity and specificity, but also have primer/probe designs into the regions of new mutations in specific SARS-CoV-2 variants, facilitates rapid design as the assay has already been clinically validated for identification of the wild-type virus. In fact, in our published article,[4] we commented on the high mutation and recombination rates of RNA viruses[5] and the need for occasional monitoring of SARS-CoV-2 sequences to identify whether mutations have developed in the regions targeted by the Northwell Health Laboratories laboratory-developed test (NWHL LDT). We also alluded to the use of additional targets with the assay to increase sensitivity in anticipation of potential viral mutations over time.

Although the NWHL LDT primer and probe set is abrogated by the HV69/70 deletion, other mutations mentioned by Nörz et al, as well as other newly described variants, that are potentially problematic. For example, the identified SARS-CoV-2 VOC B.1.1.7 (501Y.V1), B.1.351 (501Y.V2), B.1.1.28.1 (P.1), and B1.427/429 that emerged in the United Kingdom, South Africa, Brazil, and the United States, respectively,[1,3,6] appear to potentially spread more quickly than the wild type strain, likely due to extensive mutations in the spike protein. More notably, these variants share one or more additional mutations such as D614G, N501Y, and E484K. There is no doubt that this trend of new variants will continue and will also necessitate monitoring and redesign of assays in real time to adjust to the changing viral landscape. As such, further studies are needed to continue to characterize these variants as they arise.

In summary, the modification of the NWHL LDT assay for use in identification of the HV69/70 deletion as well as the additional design of a primer and probe set to detect the N501Y SNP by Nörz et al is an efficient and cost-effective design to allow for large scale screening of these two variants. This approach to SARS-CoV-2 variant identification can also be built upon as new SARS-CoV-2 mutations arise.

This model describes a potential two-tier strategy, where variant information derived from whole genome sequencing surveillance programs continues to be used to identify and characterize new variants, leading to the development of targeted molecular-based sensitive, accurate, and cost-effective diagnostic assays to continue to monitor the prevalence and clinical significance of the identified circulating SAR-CoV-2 VOC.

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