Quanterix
The Science of Precision Health

Infectious Disease

Simoa® SARS-CoV-2 N Protein Antigen Test: Robust Detection of Variants of Concern

The Simoa SARS-CoV-2 N Protein Antigen Test, developed with support from the NIH's Rapid Acceleration of Diagnostics Program (RADx) and authorized for Emergency Use by the FDA, has been demonstrated experimentally to detect SARS-CoV-2 Variants of Concern. The test, which combines the advantages of direct viral detection, fully automated high throughput sample processing, and sensitivity >100X greater than other EUA antigen tests[1], detects the SARS-CoV-2 Nucleocapsid protein. This protein is highly conserved among coronaviruses, with 90% amino acid sequence homology and fewer mutations over time, suggesting it may be less prone to genetic mutation due to selective pressure[2]. Independent testing conducted in collaboration with the NIH Variant Task Force and Emory University has confirmed the Simoa N Protein Antigen Test detects two original strains of the virus, five CDC-designated Variants of Interest, and all four Variants of Concern, as designated by the CDC as of July 8, 2021, including the highly transmissible B.1.617.2 (Delta) variant.

The Centers for Disease Control and Prevention (CDC) has classified four SARS-CoV-2 variants as "Variants of Concern" (VOC), a variant for which there is evidence of an increase in transmissibility, more severe disease (e.g., increased hospitalizations or deaths), significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures. The CDC has additionally identified seven "Variants of Interest" (VOI), which contain genetic mutations that suggest they may be prone to the same consequences as Variants of Concern[3]. While mutations in the four designated Variants of Concern are primarily concentrated in the Spike gene, which encodes the receptor binding domain that mediates viral entry into cells, mutations in the Nucleocapsid gene are also observed (Tables 1 & 2).

Table 1. Spike Protein Amino Acid Changes in the SARS-CoV-2 Variants of Concern

Variant Lineage	WHO Designation	S Protein Mutations
B.1.1.7	Alpha	N501Y, A570D, D614G, D1118H, H69-, P681H, S982A, T716I, V70-, Y144-
B.1.351	Beta	E484K, K417N, N501Y, A243-, A701V, D80A, D215G, D614G, L242-, L244-
P1	Gamma	E484K, K417T, N501Y, D138Y, D614G, H655Y, L18F, P26S, R190S, T20N, T1027I, V1176F
B.1.617.2	Delta	L452R, T478K, D614G, D950N, E156G, F157-, G142D, P681R, R158-, T19R

Table 2. Nucleocapsid Protein Amino Acid Changes in the SARS-CoV-2 Variants of Concern

Variant Lineage	WHO Designation	N Protein Mutations
B.1.1.7	Alpha	D3L, G204R, R203K, S235F
B.1.351	Beta	T205I
P1	Gamma	G204R, P80R, R203K
B.1.617.2	Delta	D63G, D377Y, G215C, R203M

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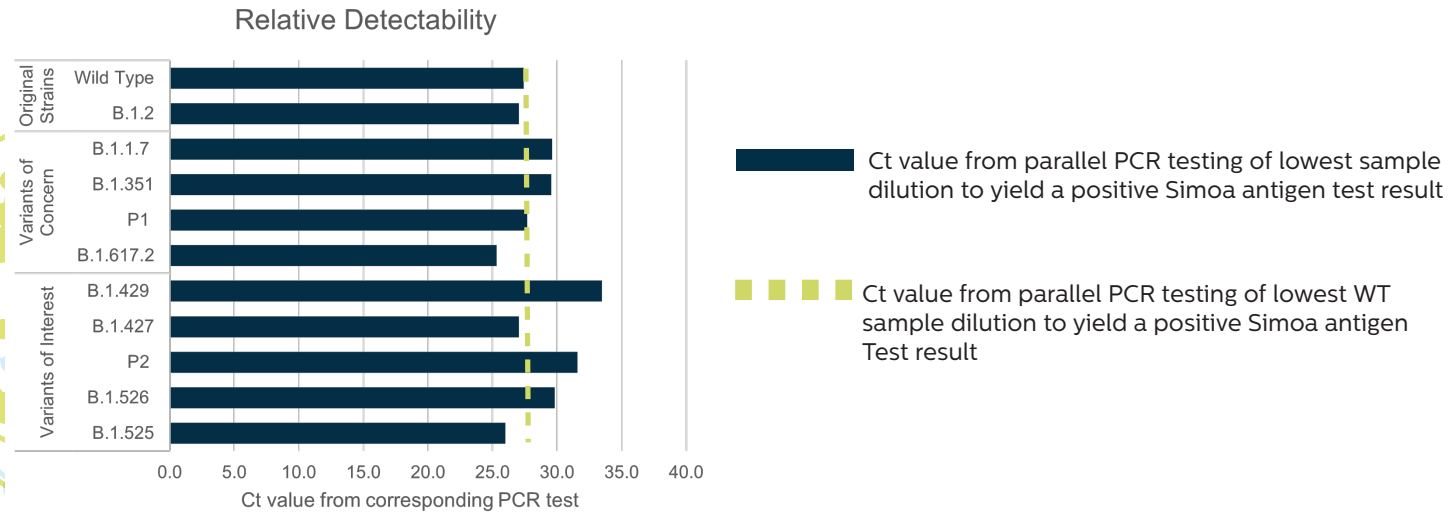
First detected in India in December of 2020, the Delta variant (B.1.617.2) represents the dominant strain source of coronavirus infections in the United States as of July 8, 2021[4]. The Delta variant is the "fastest and fittest" variant yet according to the CDC, as much as 50-60% more transmissible than the Alpha variant. Hospitalization rates for those infected with the Delta variant have are reported to be as much as 85 percent higher than that of people infected with the Alpha variant[5].

All SARS-CoV-2 diagnostic antigen tests authorized to date are designed to detect the nucleocapsid protein of the SARS-CoV-2 virus. Antigen tests rely on antibody-mediated binding to the nucleocapsid protein, which elicits signal generation. If the antibody binding epitope is known, it may be possible to predict which nucleocapsid protein amino acid changes might impair test performance, but ultimately test performance must be validated through direct testing of clinical isolates for the SARS-CoV-2 Variants.

In cooperation with the NIH Variant Task Force (VTF) and Emory University, the Simoa SARS-CoV-2 N Protein Antigen Test has been independently evaluated for detectability of five Variants of Interest, four Variants of Concern and two original viral strains. Briefly, 8-10 remnant human nasopharyngeal swab samples collected in Viral Transport Medium (VTM) and sequence verified as VOI or VOC were pooled, aliquoted, and frozen at -80C. Aliquots were subsequently thawed, and a dilution series prepared in VTM. Quality Control of pools and dilutions was performed by extracting RNA and conducting RT-PCR for detection of the N gene (N2 CDC primers) and endogenous control. Ct values from RT-PCR testing were recorded for each dilution. Dilutions were sent by the VTF to Emory University for independent testing with the Simoa SARS-CoV-2 N Protein Antigen Test. In a separate experiment, a single clinical isolate sequence verified by Emory University as the Delta variant (B.1.617.2) was diluted 1:10 and tested with the Simoa SARS-CoV-2 Antigen Test, and by RT-PCR for detection of the N gene (N1 CDC primers). The consolidated results from these two studies are shown in Figure 1, plotting the Ct value generated in RT-PCR testing of the highest dilution that yielded a positive result with the Simoa Antigen Test. Note that for the Delta variant (B.1.617.2) clinical isolate, only one dilution was tested.

Figure 1: Simoa SARS-CoV-2 N Protein Antigen Test Detection of Variants of Interest & Concern



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Taken together, these results demonstrate robust detection of sequence-confirmed clinical isolates of five of the seven CDC-designated Variants of Interest and all CDC-designated Variants of Concern with the Simoa SARS-CoV-2 N Protein Antigen Test, confirming the utility of this test for detection of SARS-CoV-2 variant lineages in clinical specimens.

References:

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2. Dutta et al., The Nucleocapsid Protein of SARS-CoV-2: A Target for Vaccine Development; Journal of Virology, 2020 Jul; 94(13)

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4. CDC COVID Data Tracker, Updated July 8, 2021

5. Sheikh A., et al, SARS-CoV-2 Delta VOC in Scotland: demographics, risk of hospital admission, and vaccine effectiveness; Lancet; Vol 397, June 26, 2021

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