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Customer Bulletin

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CLINITEST Rapid COVID-19 Antigen Test
CLINITEST Rapid COVID-19 Antigen Self-Test

Sequence Analysis of Nucleocapsid Protein from SARS-CoV-2 Variants

Dear Valued Customer,

The purpose of this communication is to provide you with more information regarding the SARS-CoV-2 variants.

Background: Genomic sequence analysis of SARS-CoV-2 isolates from around the world shows distinct patterns relative to originally isolated strains. This has led to the classification of unique variants of the virus that contain mutations in the virus genome including the N gene that codes for the nucleocapsid protein that is the target of the CLINITEST® Rapid COVID-19 Antigen Test*. Classification of variants differs between health organizations and depends on several factors including prevalence in specific regions, however there is general agreement which strains are the most significant.[1-3] The variants listed in the table below are currently classified as variants of concern (VOC) or variants of interest (VOI) by the World Health Organization.[4] Variants of concern are associated with increased transmissibility and virulence and have demonstrated a negative impact on some diagnostic tests and therapies. Variants of interest have mutations that are indicative of similar outcomes to variants of concern but have not undergone a comparative analysis. The table below includes ten variant strains and shows their classification, where they were first identified and the mutations in the nucleocapsid protein. Analysis of sequence data from these variants is described below and reveals that no more than four mutations per variant are detected in the 419 amino acid nucleocapsid protein.

Data: Nucleocapsid is a 419 amino acid protein encoded by the N gene found downstream of the spike (S), envelope (E) and membrane (M) genes. The reported mutations in the N protein relative to a reference strain are listed int the table below for each variant.
BLAST analysis of protein sequences in the National Center for Biotechnology Information (NCBI)[5] database (query SARS-CoV-2, taxid 2697049, output 1,000 sequences) shows that the mutated residues in each variant sequence share ≥98% sequence identity with other fully sequenced nucleocapsid proteins. An amino acid sequence alignment (generated with Clustal O[6]) between a reference SARS-CoV-2 isolate (hCOV-19/Wuhan/WIV04/2019) and sequences of from the variant strains was also performed. Comparison of these sequences shows ≥96% similarity between variants.

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Table of SARS-CoV-2 Variants.

	WHO Label	Lineage	Alternate Name(s)	First Identified	Nucleocapsid Mutations	References
Variant of Concern	Alpha	B.1.1.7	VOC202012/01, 20I/501Y.V1	United Kingdom	D3L R203K* G204R* S235F	7-9
	Beta	B.1.351	VOC202012/02 20H/501Y.V2	South Africa	T205I	8-9
	Gamma	P.1	B.1.1.28.1 VOC202101/02 20J/501Y.V3	Brazil	P80R R203K* G204R*	8-10
	Delta[a]	B.1.617.2	N/A	India	D63G R203M D377Y	8-9
Variant of Interest	Epsilon[b]	B.1.427 B.1.429	20C/S.452R, CAL.20C/L452R	California, United States	T205I	8-9, 11
	Iota[c]	B.1.526	N/A	New York, United States	P199L M234I	8, 12, 13
		B.1.526.1			T205I M234I	
		B.1.526.2			P13L S202R	
	Zeta	P.2	B.1.1.28.3 20B/S.484K	Brazil	A119S R203K G204R M234I	8,9,10
	None	B.1.616[d]	20C	France	T325I	9
	Eta	B.1.525	20C	United Kingdom, Nigeria	A12G T205I	9
	Theta	P.3	B.1.1.28.3	Philippines, Japan	R203K G204R	9
	Kappa	B.1.617.1	N/A	India	R203M D377Y	8-9

[a] Classified as VOI by US Centers for Disease Control. [b] Classified as VOC by US CDC. [c] Strain listed as VOI by WHO and US CDC and variant under monitoring by European CDC. [d] Strain only listed by European CDC as VOI. * Mutations not originally associated with these variants. N/A = not applicable.

Conclusion: No more than 4 amino acid changes out of the 419 possible have been observed in the nucleocapsid sequence from significant SARS-CoV-2 variant strains. BLAST analysis shows that the mutated residues in the amino acid sequence share ≥98% sequence identity with fully sequenced nucleocapsid proteins. This suggests that the CLINITEST® Rapid COVID-19 Antigen Test is unlikely to be impacted by any of the SARS-CoV-2 variants.

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