Updated March 2021 – QIAGEN response to the SARS-CoV-2 variants with increased infectivity

It is well established that RNA viruses frequently mutate due to erroneous or ineffective replication of the viral genome (1). Coronaviruses, including SARS-CoV-2, sustain fewer mutations than most RNA viruses because they encode an enzyme that corrects some of the errors made during replication. In most cases, the fate of a new mutation in the viral genome is determined by natural selection. Those that confer a competitive advantage for viral replication, transmission, or escape from immunity will increase in frequency, and those that reduce viral fitness tend to be removed from the population of circulating viruses. However, mutations can also increase and decrease in frequency due to chance events. A variant is defined by one or more mutations that differentiate it from the reference variant and other variants in circulation. As expected, multiple variants of SARS-CoV-2 have been documented globally since the start of the COVID-19 pandemic.

SARS-CoV-2 variant classification

Although multiple viral variants have been detected throughout the pandemic, most appear to have little if any biological significance. However, since late 2020 a small number of variants have been classified as variants of interest (VOI) or variants of concern (VOC) by international health organizations like WHO (2), CDC (3) and ECDC (4).

Variants of concern (VOC) (2,3) are defined as those for which evidence has been found of an increase in transmissibility, more severe disease (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.

Variants of interest (VOI) (3) are defined as those containing mutations that have been associated with changes to receptor binding, reduced neutralization by antibodies generated against previous infection or vaccination, reduced efficacy of treatments, potential diagnostic impact, or predicted increase in transmissibility or disease severity.

***in silico* assessment of the impact of mutations present in VOC and VOI**

To address the possible impact of VOC and VOI mutations on assay performance, QIAGEN continuously assesses available sequences available from public databases (GISAID and GenBank) by mapping primer designs of all available products against the mutated sequences of identified variants. This analysis allows for any potential impact on sensitivity to be immediately recognized for any of the QIAGEN assays currently used in the fight against COVID-19: the QIAstat-Dx® Respiratory SARS-CoV-2 Panel*, the NeuMoDx™ SARS-CoV-2 Assay*, and the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay*.

Upon the *in silico* analysis of available sequences of the VOC and VOI designated by the WHO (2), the CDC(3) and the ECDC(4) in their epidemiological updates (listed on the table below), we conclude that none of the recorded mutations present in the listed strains affects the sensitivity of the QIAGEN assays detecting SARS-CoV-2 to date (5).

Type of variant (VOC/VOI)		Pango lineage	Name (Nextsatrin clade)	Key mutations	First detected	Known attributes	Impact on sensitivity of SARS-CoV-2 detection by		
CDC	WHO/ ECDC						QIAstat-Dx Respiratory SARS-CoV-2 Panel	NeuMoDx SARS-CoV-2 Assay	NeuMoDx Flu A-B/RSV/ SARS-CoV-2 Vantage Assay
VOC	VOC	P.1	20J/501Y.V3	(Spike) K417N/T, E484K, N501Y, D614G	Japan/Brazil	Moderate impact on neutralization by EUA monoclonal antibody therapeutics Reduced neutralization by convalescent and post-vaccination sera	No impact	No impact	No impact
VOC	VOC	B.1.351	20H/501. V2 (also VOC 202012/02)	(Spike) K417N, E484K, N501Y, D614G	South Africa	~50% increased transmission Moderate impact on neutralization by EUA monoclonal antibody therapeutics Moderate reduction on neutralization by convalescent and post-vaccination sera	No impact	No impact	No impact
VOC	VOI	B.1.427	20C/S:452R	(Spike) L452R, D614G	US-California	~20% increased transmissibility Significant impact on neutralization by some, but not all, EUA therapeutics Moderate reduction in neutralization using convalescent and post-vaccination sera	No impact	No impact	No impact
VOC	VOI	B.1.429	20C/S:452R	(Spike) S13I, W152C, L452R, D614G	US-California	~20% increased transmissibility Significant impact on neutralization by some, but not all, EUA therapeutics Moderate reduction in neutralization using convalescent and post-vaccination sera	No impact	No impact	No impact
VOI	VOI	B.1.525	20C	**Spike**: A67V, Δ69/70, Δ144, E484K, D614G, Q677H, F888L **ORF1b**: P314F **ORF1a**: T2007I **M**: I82T **N**: A12G, T205I **5'UTR**: R81C	USA (New York)/ United Kingdom and Nigeria	Potential reduction in neutralization by monoclonal antibody treatments Potential reduction in neutralization by convalescent and post-vaccination sera	No impact	No impact	No impact

Type of variant (VOC/VOI)		Pango lineage	Name (Nextsatrin clade)	Key mutations	First detected	Known attributes	Impact on sensitivity of SARS-CoV-2 detection by		
CDC	WHO/ ECDC						QIAstat-Dx Respiratory SARS-CoV-2 Panel	NeuMoDx SARS-CoV-2 Assay	NeuMoDx Flu A-B/RSV/ SARS-CoV-2 Vantage Assay
VOI	VOI	B.1.1.28.2 (alias P.2)	20B/S.484K	**Spike**: E484K, D614G, V1176F **ORF1a**: L3468V, L3930F **ORF1b**: P314L **N**: A119S, R203K, G204R, M234I **5'UTR**: R81C	Brazil	Potential reduction in neutralization by monoclonal antibody treatments Potential reduction in neutralization by convalescent and post-vaccination sera	No impact	No impact	No impact
Not reported	VOI	B.1.1.28.3 (alias P.3)	Not yet assigned	141-143 deletion, E484K, N501Y and P681H	Philippines and Japan	N/A	No impact	No impact	No impact
VOI	VOI	B.1.526 (with E484K or S477N)	20C	**Spike**: (L5F†), T95I, D253G, (S477N†), (E484K†), D614G, (A701V†) **ORF1a**: L3201P, T265I, Δ3675/3677 **ORF1b**: P314L, Q1011H **ORF3a**: P42L, Q57H **ORF8**: T11I **5'UTR**: R81C	USA – New York	Potential reduction in neutralization by monoclonal antibody treatments Potential reduction in neutralization by convalescent and post-vaccination sera	No impact	No impact	No impact
Not reported	VOI	B.1 descendant with 9 mutations	20C	G142 deletion; D66H, Y144V, D215G, V483A, D614G, H655Y, G669S, Q949R and N1187D	France (Brittany)	N/A	No impact	No impact	No impact

The majority of the listed variants above concentrate the highest incidence of genetic variability in the S-gene that encodes for the Spike protein. None of the assays included in the QIAGEN products target the S-gene to detect SARS-CoV-2. The few mutations that occur within the nucleocapsid and other ORF regions are not predicted to alter the annealing efficiency of the oligonucleotides used in the QIAGEN assays.

	QIAstat-Dx Respiratory SARS-CoV-2 Panel	NeuMoDx SARS-CoV-2 Assay	NeuMoDx Flu A-B/RSV/SARS-CoV-2
Genomic regions targeted by the QIAGEN products	E-gene & Orf1ab gene	N-gene & Nsp2 gene	Nsp2 gene
Relevant mutations identified in current variants	Variant B.1.525 contains one identified mismatch in gene E assay binding region This mutation located in a non-critical position and no impact on annealing efficiency or assay performance is predicted	Variant B.1.1.7 contains one identified mismatch in gene Nsp2 assay binding region. This mutation located in a non-critical position and no impact on annealing efficiency and or assay performance has been proven.	

Genetic variability alignment and impact assessment using all sequences available on public databases

In addition to this assessment of publicly identified VOCs and VOIs of SARS-CoV-2, QIAGEN also performs a thorough surveillance activity that includes the analysis of all sequences of SARS-CoV-2 available from public databases (GISAID and GenBank) to ensure designs utilized on available products remain unaffected by mutations found with any significant frequency. Regulatory Authorities/ Agencies recommendations are strictly followed to perform both in-silico and in-vitro testing when required.

Between February 22 to March 21, 2021, 231,641 SARS-CoV-2 sequences were uploaded onto the public databases (GISAID and GenBank). After performing the analysis following our published methodology (6) for all QIAGEN assays currently used in the fight against COVID-19: the QIAstat-Dx Respiratory SARS-CoV-2 Panel*, the NeuMoDx SARS-CoV-2 Assay*, and the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay*, it was concluded that over 99% of the genomes analyzed contained either no mismatches or no mismatches located in critical positions of the oligonucleotide binding sites.

The small number of sequences (<1%) that contained any mutations in critical positions of the binding sites of the oligonucleotides have been assessed based on their prevalence and increased frequency. The few mutations for which some level of criticality has been encountered will be tested in vitro to ensure the sensitivity of the QIAGEN assays is kept as specified.

From the onset of the novel coronavirus outbreak, QIAGEN's dedicated global teams have been working around the clock to support the worldwide fight against COVID-19. We will continue with our genetic variation surveillance on a regular basis. Please do not hesitate to reach out to your local QIAstat-Dx or NeuMoDx specialist with questions.

References:

1. Shen Z, et al. (2020) Genomic Diversity of Severe Acute Respiratory Syndrome-Coronavirus 2 in Patients With Coronavirus Disease 2019. Clin Infect Dis **71**, 713-20.
2. World Health Organization. Weekly epidemiological update on COVID-19- 23 March 2021. https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19—23-march-2021
3. Centers for Disease Control and Prevention. SARS-CoV-2 Variants Classifications and Definitions. https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html#Concern (accessed March 26, 2020)
4. European Centre for Disease Prevention and Control. (2020) Risk related to spread of new SARSCoV-2 variants of concern in the EU/EEA. https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-risk-related-to-spread-of-new-SARS-CoV-2-variants-EU-EEA.pdf
5. Penarrubia L. et al. (2021) In Response to: Multiple assays in a real-time RT-PCR Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) panel can mitigate the risk of loss of sensitivity by new genomic variants during the COVID-19 outbreak. Int J Infect Dis https://doi.org/10.1016/j.ijid.2021.01.049
6. Peñarrubia, Luis. et al. (2020) Multiple assays in a real-time RT-PCR SARS-CoV-2 panel can mitigate the risk of loss of sensitivity by new genomic variants during the COVID-19 outbreak. Int J Infect Dis. **97**, 225-9

* Products and product claims may differ from country to country based on regulations and approvals. Contact your country representative for further details.

† Detected in some sequences but not all.