Updated March 2022 – 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), ECDC (4) and UKHSA (5).

Variants of concern (VOC) (2-5) 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) (2-4) / **variant under investigation (VUI)** (5) 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/VUI

To address the possible impact of VOC and VOI/VUI mutations on assay performance, QIAGEN continuously assesses available sequences available from public databases (GISAID) 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 QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel*, the NeuMoDx™ SARS-CoV-2 Assay*, the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay*, and the *artus*® SARS-CoV-2 Prep&Amp UM Kit*.

Upon the in silico analysis of available sequences of the VOC and VOI/VUI designated by the WHO (2), the CDC (3), the ECDC (4), and UKHSA (5) 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 (6).

Type of variant (VOC/VOI/VUI)									Impact on sensitivity of SARS-CoV-2 detection by				
CDC	ECDC	UK Health Security Agency	WHO	WHO label	Pango lineage	Name (Nextstrain clade)	Key Spike Mutations	First detected	QIAstat-Dx Respiratory SARS-CoV-2 Panel	QIAstat-Dx SARS-CoV-2 /Flu A/B/ RSV Panel	NeuMoDx SARS-CoV-2 Assay	NeuMoDx Flu A-B/ RSV/SARS-CoV-2 Vantage Assay	*artus* SARS-CoV-2 Prep&Amp UM Kit
Under monitoring	n/a	VOC-20DEC-01	VOC	Alpha	B.1.1.7 lineage, consisting of sublineages: Q.1 Q.2 Q.3 Q.4 Q.5 Q.6 Q.7 Q.8	20I/501Y.V1 (also VOC 202012/01)	Δ69/70, Δ144, (E484K†), (S494P†), N501Y, A570D, D614G, P681H, T716I, S982A, D1118H (K1191N†)	United Kingdom (Sept 2020)	No impact	No impact	No impact	No impact	No impact
Under monitoring	VOC	VOC-20DEC-02	VOC	Beta	B.1.351 lineage, consisting of sublineages: B.1.351.1 B.1.351.2 B.1.351.3 B.1.351.4 B.1.351.5	20H/501.V2 (also VOC 202012/02)	D80A, D215G, Δ241/242/243, K417N, E484K, N501Y, D614G, A701V	South Africa (May 2020)	No impact	No impact	No impact	No impact	No impact
Under monitoring	VOC	VOC-21JAN-02	VOC	Gamma	P.1 lineage, consisting of sublineages: P.1.1, P.1.2, P.1.3, P.1.4, P.1.5, P.1.6, P.1.7, P.1.7.1, P.1.8, P.1.9, P.1.10, P.1.10.1, P.1.10.2, P.1.11, P.1.12, P.1.12.1, P.1.13, P.1.14, P.1.15, P.1.16, P.1.17, P.1.17.1, P.1.7.1	20J/501Y.V3 (also VOC 202101/02)	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I	Japan/Brazil (Nov 2020)	No impact	No impact	No impact	No impact	No impact
VOC	VOC	VOC-21APR-02	VOC	Delta	B.1.617.2 lineage, consisting of sublineages: B.1.617.2 AY.1 – AY.133	21A, 21I, 21J	T19R, (V70F†), T95I, G142D, E156-, F157-, R158G, (A222V†), (W258L†), (K417N†), L452R, T478K, D614G, P681R, D950N	India (Dec 2020)	No impact	No impact	No impact	No impact	No impact
VOC	n/a	VUI-21OCT-01	n/a	Delta	AY.4.2‡	-	L452R, T478K, D614G, P681R, A222V, Y145H	UK (Jun 2021)	No impact	No impact	No impact	No impact	No impact
Under monitoring	n/a	VUI-21JUL-01	VOI	Mu	B.1.621 lineage, including B.1.621.1, B.1.621.2, BB.1 and BB.2 sublineage	21H	R346K, E484K, N501Y, D614G, P681H	Colombia (Jan 2021)	No impact	No impact	No impact	No impact	No impact
n/a	n/a	n/a	VOI	Lambda	C.37 lineage, including C.37.1 sublineage	21G	L452Q, F490S, D614G	Peru (Dec 2020)	No impact	No impact	No impact	No impact	No impact

Type of variant (VOC/VOI/VUI)									Impact on sensitivity of SARS-CoV-2 detection by				
CDC	ECDC	UK Health Security Agency	WHO	WHO label	Pango lineage	Name (Nextstrain clade)	Key Spike Mutations	First detected	QIAstat-Dx Respiratory SARS-CoV-2 Panel	QIAstat-Dx SARS-CoV-2 /Flu A/B/ RSV Panel	NeuMoDx SARS-CoV-2 Assay	NeuMoDx Flu A-B/ RSV/SARS-CoV-2 Vantage Assay	*artus* SARS-CoV-2 Prep&Amp UM Kit
Under monitoring	n/a	VUI-21JAN-01	n/a	Zeta	B.1.1.28.2 (alias P.2)	20B/S.484K	E484K, (F565L†), D614G, V1176F	Brazil (Apr 2020)	No impact	No impact	No impact	No impact	No impact
VOC	VOC	VOC-21NOV-01	VOC	Omicron	B.1.1.529, including BA.1, BA.1.1, BA.2 and BA.3 sublineage	21K	A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211-212, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F	Multiple countries (Nov 2021)	No impact	No impact	No impact	No impact	No impact

† Detected in some sequences but not all.

‡ AY.4.2 is a sub-lineage within Delta that has been assigned as a distinct VUI.

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. Most of the few mutations that occur within the nucleocapsid ORF1ab and envelope regions are not predicted to alter the annealing efficiency of the oligonucleotides used in the assays. One mutation, however, characteristic to Lambda variant (C.37 lineage including C.37.1 sublineage) and **Omicron variant** (B.1.1.529 lineage including BA.1, BA.1.1, BA.2 and BA.3 sublineage), was assessed to potentially impact detection of N1 target of *artus* SARS-CoV-2 Prep&Amp UM Kit. Subsequent in vitro testing showed no impact on performance of the N1 target assay for this mutation.

	QIAstat-Dx Respiratory SARS-CoV-2 Panel	QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel	NeuMoDx SARS-CoV-2 Assay	NeuMoDx Flu A-B RSV/SARS-CoV-2 Vantage Assay	*artus* SARS-CoV-2 Prep&Amp UM Kit
Genomic regions targeted by the QIAGEN products	E-gene & Orf1ab gene	E-gene & Orf1ab gene	N- gene & Nsp2 gene	Nsp2 gene	N1 and N2 gene
Relevant mutations identified in current variants	• AY.85 sublineage of Delta variant contains one identified mismatch in E gene oligonucleotide binding region. This mutation is located in a non-critical position and no impact on annealing efficiency or assay performance is predicted. • AY.4.4 and AY.22 sublineages of Delta variant contain one identified mismatch in Orf1ab gene oligonucleotides binding region. This mutation is located in a critical position and its impact on assays performance has been checked experimentally. No decrease in assays sensitivity has been observed. • AY.46.3 sublineage of Delta variant contains one identified mismatch in Orf1ab gene oligonucleotide binding region.This mutation is located in a non-critical position and no impact on annealing efficiency or assay performance is predicted. • AY.102.2 sublineage of Delta variant contain one identified mismatch in E gene oligonucleotides binding region. This mutation is located in a critical position and its impact on assays performance has been checked experimentally. No decrease in assays sensitivity has been observed		• Alpha variant (B.1.1.7 lineage), Q.1, Q.2, Q.3, Q.4, Q.5, Q.6, Q.7 and Q.8 sublineage contains one identified mismatch in gene Nsp2 oligonucleotide binding region. This mutation is located in a non-critical position and no impact on annealing efficiency or assay performance is predicted. • Q.5 – sublineage of Alpha variant contains one identified mismatch in gene N assay binding region. This mutation is located in a non-critical position and no impact on annealing efficiency or assay performance is predicted. • AY.114 sublineages of Delta variant contain one identified mismatch in gene Nsp2 oligonucleotide binding region. This mutation is located in a non-critical position and no impact on annealing efficiency or assay performance is predicted. • AY.123.1 sublineage of Delta variant contain one identified mismatch in gene Nsp2 oligonucleotide binding region. This mutation is located in a non-critical position and no impact on annealing efficiency or assay performance is predicted. • AY.5, AY.5.1, AY.5.2, AY.5.4, AY.5.5 and AY.97 – sublineage of Delta variant contains one identified mismatch in gene N assay binding region. This mutation is located in a non-critical position and no impact on annealing efficiency or assay performance is predicted.	• Lambda variant (C.37 lineage including C.37.1 sublineage) and the **Omicron variant** (B.1.1.529, including BA.1, BA.2 and BA.3 sublineage) contains one identified mismatch in gene N1 assay binding region. This mutation is located in a critical position and its impact on assays performance has been checked experimentally. No decrease in assays sensitivity has been observed. • Q.6 - sublineage of Alpha variant contains one identified mismatch in gene N2 assay binding region. This mutation is located in a non-critical position and no impact on annealing efficiency or assay performance is predicted. • AY.47 sublineage of the Delta variant contains one identified mismatch in gene N1 assay binding region. This mutation is located in a non-critical position and no impact on annealing efficiency or assay • AY.43.6 sublineage of The Delta variant contains one identified mismatch in gene N1 assay binding region. This mutation is located in a non-critical position and no impact on annealing efficiency or assay performance is predicted. • AY.91.1 sublineage of the Delta variant contains one identified mismatch in gene N1 assay binding region. This mutation is located in a critical position and its impact on assays performance has been checked experimentally. No decrease in assays sensitivity has been observed.

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

In addition to this assessment of publicly identified VOCs and VOIs/VUIs of SARS-CoV-2, QIAGEN also performs a thorough surveillance activity. This includes the analysis of all sequences of SARS-CoV-2 available from public databases (GISAID) 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.

After performing the analysis following our published methodology (6,7) for all QIAGEN assays currently used in the fight against COVID-19: the QIAstat-Dx Respiratory SARS-CoV-2 Panel, the QIAstat-Dx SARS-CoV-2/Flu A/B/RSV Panel, the NeuMoDx SARS-CoV-2 Assay, the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay, and the *artus* SARS-CoV-2 Prep&Amp UM Kit, it was concluded that given the low prevalence of the mutations in critical positions and the fact that critical mutations in both amplicons were present in only 169 genomes, mentioned kits are estimated to keep 100% of inclusivity against the SARS-CoV-2 genome.

The small number of sequences 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, NeuMoDx, or *artus* 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. **https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/** (accessed March 4, 2022)
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** (accessed March 4, 2022)
4. European Centre for Disease Prevention and Control (2021). Mutation of SARS-CoV-2 - current variants of concern **https://www.ecdc.europa.eu/en/covid-19/variants-concern** (accessed March 4, 2022)
5. UK Health Security Agency. SARS-CoV-2 variant data update, England, Version 23 (published on February 25, 2022).
6. Peñarrubia 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**
7. 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.

Trademarks: QIAGEN®, Sample to Insight®, QIAstat-Dx®, *artus*®, NeuMoDx™ (QIAGEN Group). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

PROM-17719-012 03/2022 © QIAGEN 2022, all rights reserved.