

Current altona Diagnostics SARS-CoV-2 test kits reactivity analyses in response to mutations

Section 1 does focus specifically on the S gene mutations as these are of the highest public concern at the moment. Not only due to the possible implications for molecular diagnostic assays but even more so due to potential devastating effects on vaccine efficiency and possible immune evasion of these variants.

Section 2 shows the summarized results from our latest bioinformatical analysis which were performed using all known sequence data (including all variants of concern) from different online sources (Table 1).

Section 1: Variants of Concern and Other Spike Mutations

The SARS-CoV-2 variant of concern (VOC-202012/01, formerly known as VUI-202012/01 and also known as lineage B.1.1.7 or 20B/501Y.V1, England, United Kingdom) is defined by variant S gene mutations:

- deletion 69-70
- deletion 144
- N501Y
- A570D
- D614G
- P681H
- T716I
- S982A
- D1118H

Other known spike protein mutations are:

- Y453F
- N439K
- I692V
- M1229I
- A222V
- D80Y
- A626S
- A701V
- S98F
- S477N
- deletion 21765-21770
- deletion 21991-21993
- A23063T
- C23271A
- C23604A
- V1176F
- C23709T
- T24506G
- G24914C
- E484K
- K417N
- D215G
- E484K

None of these mutations does impact the performance of the S gene detection system included in the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 and the AltoStar® SARS-CoV-2 RT-PCR Kit 1.5. The target region of the S gene assays contained in the RealStar and AltoStar assay is located in the region coding for the domain 2 of the spike protein.

Section 2: *In Silico* Reactivity Analysis

Latest (January 11, 2021) inclusivity data were collected and *in silico* analysis was updated with the newly published sequences and data (see Table 1).

Inclusivity (*In silico* analysis for **258,701** whole genome sequences of SARS-CoV-2 published via GISAID e.V. (www.gisaid.org) and **45,259** whole genome sequences published via National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) as of January 11, 2021 for the E gene and the S gene target included in the RealStar® and AltoStar® SARS-CoV-2 RT-PCR Kits.

303,960 whole genome sequences		Homology	Comment
E gene	Forward Primer	303,629 sequences: 100%	328 sequences: 96.2% (1 mismatch) 3 sequences: 92.3% (2 mismatches)
	Reverse Primer	303,717 sequences: 100%	242 sequences: 95.5% (1 mismatch) 1 sequence: 90.9% (2 mismatches)
	Probe	303,707 sequences: 100%	252 sequences: 95.7% (1 mismatch) 1 sequence: 91.3% (2 mismatches)
S gene	Forward Primer	302,614 sequences: 100%	1,338 sequences: 95.2% (1 mismatch) 8 sequences: 90.5% (2 mismatches)
	Reverse Primer	300,399 sequences: 100%	3553 sequences: 95.5% (1 mismatch) 8 sequences: 90.1% (2 mismatches)
	Probe	301,612 sequences: 100%	2,340 sequences: 96.3% (1 mismatch) 7 sequences: 92.6 % (2 mismatches) 1 sequence: 85.2%*(4 mismatches)

* The sequence (accession ID EPI_ISL_415593, GISAID) showed 4 mismatches in the S gene probe binding site. This sequence was published on March 10, 2020 originating from Washington, USA. Since then none of the published sequences showed that many mismatches again. The sequence was commented by the authors "Caution. Stretches of NNNs (1.74 % of overall sequence)", indicating not ideal sequencing quality, the impact on the S gene specific oligonucleotides has therefore not been investigated.

Depending on the position, mutation events leading to ≤ 2 mismatch/es in a single oligonucleotide sequence are very unlikely to have any significant negative effect on the performance of the assay. All such sequences (≤ 2 mismatch/es) tested in wet lab experiments in the cause of the post market surveillance activities for the RealStar® SARS-CoV-2 RT-PCR Kit 1.0 so far confirmed that the performance was not affected by such mutations. With the exception of one unique sequence none of the other analyzed sequences showed mismatches in more than one oligonucleotide and none of the mismatching sequences showed mismatches with both specific detection systems (E gene and S gene), hence reactivity of the specific oligonucleotides included in the RealStar® and AltoStar® SARS-CoV-2 RT-PCR Kits is not expected to be affected.