Current altona Diagnostics SARS-CoV-2 test kits reactivity analyses in response to mutations (September 20, 2021)

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 listed in section 1) from different online sources (Table 2).

Sequence analysis is performed every week and the summarized results are published monthly.

Section 1: Variants of concern and other spike mutations

Several SARS-CoV-2 lineages originating from different regions of the world have been described so far. Among those are the variants listed in Table 1:

Table 1: SARS-CoV-2 variants

WHO Label	PANGOLIN Lineage	GISAID Lineage	Likely Origin
VOC Alpha	B.1.1.7	GRY	United Kingdom(UK)
VOC Beta	B.1.351	GH/501Y.V2	South Africa
VOC Gamma	P1	GR/501Y.V3	Brazil
VOC Delta	B.1.617.2 + AY.1 + AY.2	G/478K.V1	India
VOI Epsilon	B.1.427 + B.1.429	GH/452R.V1	California, US
VOI Zeta	P.2	GR/484K.V2	Brazil
VOI Eta	B.1.525	G/484K.V3	UK/Nigeria
VOI Theta	P.3	GR/1092K.V1	Philippines
VOI Iota	B.1.526	GH/253G.V1	USA
VOI Kappa	B.1.617.1	G/452R.V3	India
VOI Lambda	C37	GR/452Q.V1	Peru
VOI Mu	B.1.621	GH	Colombia

The spike protein mutations characteristic for the above-mentioned variants are located in domain 1 of the protein. **None of these mutations does impact the performance of the S gene detection system included in the RealStar®, FlexStar® and AltoStar® kits for detection of SARS-CoV-2.** The target region of the S gene assays contained in these products is located in domain 2 of the spike protein.

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Section 2: *In silico* reactivity analysis

Latest (September 12, 2021) inclusivity data were collected and *in silico* analysis was updated with the newly published sequences and data (see Table 2).

Table 2: Inclusivity (*In silico* analysis for **2,453,908** whole genome sequences of SARS-CoV-2 published via GISAID e.V. (www.gisaid.org) as of September 12, 2021 and **501,400** whole genome sequences published via National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) as September 12, 2021 for the E gene and the S gene target included in the RealStar®, FlexStar® and AltoStar® kits for detection of SARS-CoV-2.

2,955,308 whole genome sequences		**Sequences showing 100% homology**	**Sequences showing mismatches (number of mismatches)**
E gene	Forward Primer	2,949,844	5,457 (1) 7 (2)
	Reverse Primer	2,952,825	2,477 (1) 5 (2) 1 (3) **
	Probe	2,953,689	1,611 (1) 4 (2)
S gene	Forward Primer	2,938,606	16,534 (1) 151 (2)
	Reverse Primer	2,935,156	20,098 (1) 54 (2)
	Probe	2,944,066	11,201 (1) 37 (2) 3 (3) * 1 (4)

* 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.

** The sequence (accession MW584978.1) showed 3 mismatches in the E gene reverse primer binding site. This sample was collected on April 03, 2020 and published on February, 2021 originating from Cleveland, USA. Since then, none of the published sequences showed that many mismatches again.

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®, FlexStar® and AltoStar® kits for detection of SARS-CoV-2 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®, FlexStar® and AltoStar® kits for detection of SARS-CoV-2 is not expected to be affected.

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