

**In silico and/or experimental analysis of the different variants applicable
to the following products:
SARS-CoV-2 Real Time PCR LAB-KIT™
BIOMAXIMA**

To whom it may concern,

SARS-CoV-2 VUI 202012/01 (Variant under investigation, year 2020, month 12, variant 01) founded in the United Kingdom (UK). The specimen date for the first COVID-19 case with the VOC 202012/01 variant in England was 20 September 2020. As of 4 January 2021, a total of 6,008 cases with this variant have been identified in England, via routine genomic surveillance. This new variant is defined by the presence of multiple mutations:

Table 1. Non-synaonymous mutation and deletions inferred to occur on the branch leading to B.1.1.7 lineage

gene	nucleotide	amino acid
ORF1ab	C3267T	T1001I
	C5388A	A1708D
	T6954C	I2230T
	11288-11296 deletion	SGF 3675-3677 deletion
spike	21765-21770 deletion	HV 69-70 deletion
	21991-21993 deletion	Y144 deletion
	A23063T	N501Y
	C23271A	A570D
	C23604A	P681H
	C23709T	T716I
	T24506G	S982A
	G24914C	D1118H
ORF8	C27972T	Q27stop
	G28048T	R52I
	A28111G	Y73C
N	28280 GAT->CTA	D3L
	C28977T	S235F

Concluded:

- o **ORF1ab gene:** Not affected by any mutation, there are no detection problems.
- o **N gene:** Analyzing the specific mutations in the *N* gene, it can be concluded that there are no detection problems.

501Y.V2 (South Africa)

This variant (named as 501Y.V2) is characterized by eight lineage-defining mutations in the spike protein, including three at important residues in the receptor-binding domain (K417N, E484K and N501Y) that may have functional significance. Mutations in *ORF1ab*, *ORF3a*, *N* and *E* genes were also reported.

This lineage emerged in South Africa after the first epidemic wave in a severely affected metropolitan area located on the coast of the Eastern Cape Province. Concluded:

- o **ORF1ab gene:** Not affected by any mutation, there are no detection problems.
- o **N gene:** One point mutation in the Forward primer. Detection is not expected to be compromised*.

* Pending experimental analysis (wet analysis) to check if PCR amplification of target *N* could be hindered.

Cluster 5 variant (North Jutland, Denmark).

Since June 2020, 214 human cases of COVID-19 have been identified in Denmark with SARS - CoV-2 variants associated with farmed minks, including 12 cases with a unique variant. This variant, referred to as the "cluster 5" variant, had a combination of mutations, or changes that have not been previously observed.

Concluded:

- o **ORF1ab gene:** Not affected by any mutation, there are no detection problems.
- o **N gene:** Not affected by any mutation, there are no detection problems.

Mutations in the 5'-end of the N gene forward primer (AAC[^] GGG)

Due to the identification of a GGG[^]AAC mutation at genome positions 28881 to 28883 that overlaps the first three bases of the 5' end of the Chinese CDC N gene forward primer, a sequence in silico analysis and/or an experimental analysis (if necessary) have been carried out.

Concluded:

- o **ORF1ab gene:** Not affected by any mutation, there are no detection problems.
- o **N gene:** Mutation in the 5'-end of the N gene forward primer (AAC[^] GGG) does not affect detection of this variant. Results showed no differences in Ct values, reaction efficiency or analytical sensitivity.

Bibliography:

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- <https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563> (Accessed: 21 /12/2020).
- Public Health England. Investigation of novel SARS-CoV-2 variant: Technical briefing 1. <https://www.gov.uk/government/publications/investigation-of-novel-sars-cov-2-variant-variant-of-concern-20201201> (Accessed 12/01/2021).
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- Tegally H et al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. medRxiv 2020. doi: 10.1101/2020.12.21.20248640.