

Gdansk, 19th July 2021

To whom it may concern,

The GeneMe considering the latest information on the mutation of the SARS-CoV-2 genome and its influence on the results of RT-PCR tests. We hereby present our Research and Development report the objective of which was to determine if the recent observed mutations in the SARS-CoV-2 affect the loss of specificity of the SAVD RT-PCR test. This research and development report summarizes our findings regarding the influence of *ORF1ab* gene mutations in SARS-CoV-2 on SAVD primers hybridization. For this purpose, bioinformatic analysis of the mutated sequences of the Coronavirus *ORF1ab* gene was performed to assess if the point mutation or deletions lay in the hybridization region of SAVD RT-PCR primers.


The SAVD by GeneMe SARS-CoV-2 Direct Rapid Detection Kit is designed for an *in vitro* identification of the Coronavirus SARS-CoV-2 in a single reaction. The presence of an innovative and patented *Pwo* polymerase and specific primers in the Kit has allowed to create the highly specific and sensitive SARS-CoV-2 rapid detection Kit. The individually designed primers are 100% compatible with the SARS-CoV-2 genomic RNA sequence of *ORF1ab* gene recommended by WHO and deposited in the NCBI database. Amplification of the targeted nucleic acids is observed by an increase of fluorescence signal during the reaction.

The GeneMe constantly cooperates with diagnostic laboratories in Poland and abroad (UK, Norway, Uganda, Mexico), regularly validating the test on clinical trials - swabs and saliva samples to make sure that the SAVD test maintains its sensitivity and specificity to the current SARS-Co-2 virus strains.

We signed below state that the SAVD RT-PCR test's ability to detect SARS-CoV-2 remains at the highest level regardless of new mutations.

Yours sincerely,


Dr Sabina Żółdowska
CQO


Dr Eng. Marta Skwarecka
Head of Research and Development Department



Research and Development

NAME: *Marta Skwarecka, Head of RD GeneMe*

DATE: *08.06.2021*

PROPOSED PRODUCT: *SAVD*

1. Title (*The title tells what has been done. Should be short (preferably up to ten words) and describe the main point of the research.*)

Detection of "Vietnamese variant" of SARS-CoV-2 virus by SAVD test.

2. Purpose and scope (*explain what the research is in a long sentence (be specific!)*)

The aim of the study is to check the universality of the SAVD test for the identification of the new variants of the SARS-CoV-2 virus. The most popular variants of the mutant SARS-CoV-2 virus were analysed in-silico: B.1.1.7 United Kingdom, B.1.351 South Africa (also known as S.501Y.V2), B.1.1.28 Brazil P1, P2, B.1.617 India and Vietnamese variant – it is an Indian (B.1.617) variant with mutations that originally belong to the U.K. (B.1.1.7). Based on current WHO information, it is a Delta variant (B.1.617.2) with additional mutations such as: K417 in the S gene (Delta + K417N).

3. Method

Date of the test:	<i>08.06.2021</i>
Place of the test:	<i>GeneMe, ul. Szybowcowa 8a, 80-298 Gdansk, Poland</i>
Test conditions (temperature, humidity):	<i>Temp: 22°C Humidity: 36%</i>
The person performing the tests:	<i>Dr Eng. Marta Skwarecka</i>
LOT of reagents analyzed:	<i>--</i>
LOT of reference reagents and trade name:	<i>--</i>

Description of the tested method:

The study consisted of:

- 1. Finding in the analysed variants of the SARS-CoV-2 virus the resulting mutations in the ORF1ab gene relative to the native strain and locating them in the genomic RNA of the virus.*
- 2. Assigning individual mutations to appropriate nucleotides.*

3. Comparison of the location of the mutated nucleotides with the location of the ORF1ab gene fragment, which is the target of the SAVD test.
4. Confirmation or exclusion of the effect of the mutation on the SAVD test identification capabilities.

4. Tested samples (enter here what samples were tested)

Sample number	Name	Supplier	Producer (as commercial material)	Concentration (as commercial material)
1.	n/a	n/a	n/a	n/a
2.	n/a	n/a	n/a	n/a

5. Results (tables with results, tables with comparative results, charts, data repository)

Table 1 shows the popular variants of the SARS-CoV-2 virus along with the changed nucleotides and compared with the target sequence of the SAVD test.

Table 1. Mutations in the ORF1ab gene of popular variants of the SARS-CoV-2 virus and their impact on the possibility of identification with the SAVD test.

Virus variant	Country of origin (emergence)	Amino-acid mutation	Nucleotide mutation	Detection with the SAVD test
Reference Strain: Wuhan-Hu-1, nCoV	China	-	-	Yes
B.1.617	India	synonymous mutation	C3037T	Yes
		synonymous mutation	C3457T	
		Thr1567Ile	C4965T	
		synonymous mutation	G8491A	
		Thr3646Ala	A11201G	
		Pro4715Leu	C14408T	
		synonymous mutation	G14772A	
synonymous mutation	C16134T			

			<i>Gly5530Cys</i>	<i>G16852T</i>	
			<i>Met5753Ile</i>	<i>G17523T</i>	
			<i>Lys6711Arg</i>	<i>A20396G</i>	
			<i>Ser6713Ala</i>	<i>T20401G</i>	
<i>B.1.1.28</i> <i>P1, P2</i>	<i>Brazil</i>		<i>synonymous mutation</i>	<i>T733C</i>	<i>Yes</i>
			<i>synonymous mutation</i>	<i>C2749T</i>	
			<i>Ser1188Leu</i>	<i>C3828T</i>	
			<i>Lys1795Gln</i>	<i>A5648C</i>	
			<i>synonymous mutation</i>	<i>A6319G</i>	
			<i>synonymous mutation</i>	<i>A6613G</i>	
			<i>synonymous mutation</i>	<i>C12778T</i>	
			<i>synonymous mutation</i>	<i>C13860T</i>	
			<i>Glu1264Asn</i>	<i>G17259T</i>	
			<i>synonymous mutation</i>	<i>C100T</i>	
			<i>Leu3468Val</i>	<i>T10667G</i>	
			<i>synonymous mutation</i>	<i>C11824T</i>	
	<i>Leu3930Phe</i>	<i>C12053T</i>			
<i>B.1.351</i> <i>(S.501Y.V2)</i>	<i>South Africa</i>		<i>Thr265Ile</i>	<i>C1059T</i>	<i>Yes</i>
			<i>Lys1655Asn</i>	<i>G5230T</i>	
			<i>Lys3353Arg</i>	<i>A10323AG</i>	
<i>B.1.1.7</i>	<i>UK</i>		<i>Thr1001Ile</i>	<i>C3267T</i>	<i>Yes</i>
			<i>Ala1708Asp</i>	<i>C5388A</i>	
			<i>Ile2230Thr</i>	<i>T6954C</i>	
			<i>SerGlyPhe 3675-3677 deletion</i>	<i>11288-11296 deletion</i>	

B.1.617.2	India	Pro4715Leu	14408-14410	Yes
		Pro5401Leu	16466-16468	
		Gly5063Ser	20515-20517	
B.1.617.2+ (Delta+)	Vietnamese	synonymous mutation	C3037T	Yes
		synonymous mutation	C3457T	
		Thr1567Ile	C4965T	
		synonymous mutation	G8491A	
		Thr3646Ala	A11201G	
		Pro4715Leu	C14408T	
		synonymous mutation	G14772A	
		synonymous mutation	C16134T	
		Gly5530Cys	G16852T	
		Met5753Ile	G17523T	
		Lys6711Arg	A20396G	
		Ser6713Ala	T20401G	
		Thr1001Ile	C3267T	
		Ala1708Asp	C5388A	
		Ile2230Thr	T6954C	
		SerGlyPhe 3675-3677 deletion	11288-11296 deletion	
Pro4715Leu	14408-14410			
Pro5401Leu	16466-16468			
Gly5063Ser	20515-20517			

Link to the data repository kept in the cloud: --

6. Conclusions (logical interpretation of the results (what happened, what didn't, why?), Identify the limitation of the study (why something did not work))

The presented analysis shows that none of the mutations occurring in the variants of the SARS-CoV-2 virus, i.e., B.1.1.7 United Kingdom, B.1.617 India and B.1.617.2 Delta, Delta+

Vietnamese did affect the effectiveness of the virus detection with the SAVD test. All analysed variants are fully identifiable with the SAVD test.

7. References (if there is a reference to the literature, please enter it here).

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3. F. Naveca, V. Nascimento, V. Souza et al. Phylogenetic relationship of SARS-CoV-2 sequences from Amazonas with emerging Brazilian variants harboring mutations E484K and N501Y in the Spike protein, 9 Feb 2021
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- 18.

Approved for external release by

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