Gdansk, 29th November 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 assays. We hereby present our Research and Development report the objective of which was to determine if the recently 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 the *in vitro* identification of the new coronavirus SARS-CoV-2, in a single reaction. The presence of an innovative and patented *Pwo* polymerase and specific primers in the kit has enabled the creation of a highly specific and sensitive SARS-CoV-2 rapid detection kit. The specifically designed primers are 100% compatible with the SARS-CoV-2 genomic RNA sequence of gene ORF1ab 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.

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

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.

Yours sincerely,

Dr Sabina Żołędowska,
CQO

Dr Eng. Marta Skwarecka,
Head of Research and Development Department

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GENEME	**Research and Development**

NAME: ***Marta Skwarecka, Head of RD GeneMe***

DATE ***28.11.2021***

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 different variants 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 known variants of the SARS-CoV-2 virus. The most popular variants of the mutant SARS-CoV-2 virus were analyzed in sillico: 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, B.1.429/ B.1.427 California variant (also known as epsilon variant), Vietnamese variant (Delta variant B.1.617.2 with additional mutations) and Omicron variant B.1.1.529.

3. Method

Date of the test:	--
Place of the test:	***GeneMe, ul. Kampinoska 25, 80-180 Gdansk, Poland***
Test conditions (temperature, humidity):	***Temp: 22°C*** ***Humidity: 36%***
The person performing the tests:	***Dr Eng. Marta Skwarecka***
LOT of reagents analyzed:	--
LOT of reference reagents and trade name:	--

Description of the tested method:

The study consisted of:

1. *Finding in the analyzed 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.*

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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*	
		Gly5530Cys	*G16852T*	
		Met5753Ile	*G17523T*	

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

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		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	*15452-15454*	
B.1.429, B.1.427	*California (USA)*	*Thr265Ile*	*1058-1060*	*Yes*
		Ser3158Thr	*9737-9739*	
		Ile4205Val	*12878-1280*	
		Pro314Leu	*1205-1207*	
		Pro976Leu	*3191-3193*	
		Asp1183Tyr	*3812-3814*	
B.1.1.529	*South Africa*	*Lys38Arg (nsp3)*	*2568-2571*	*Yes*
		Val1069Ile (nsp3)	*5662-5664*	
		Δ1265 (nsp3)	*6249-6252*	
		Leu1266Ile (nsp3)	*6253-6255*	
		Ala1892Thr(nsp3)	*8131 - 8133*	
		Thr492Ile (nsp4)	*9766-9768*	

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		Pro132His (nsp5)	*10186-10188*	
		Δ105-107 (nsp6)	*11023-11031*	
		Ala189Val (nsp6)	*11275-11277*	
		Pro323Leu (nsp12)	*15949-15951*	
		Ile42Val (nsp14)	*18940-18942*	

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.1.351 South Africa (also known as S.501Y.V2), B.1.1.28 Brazil P1, P2, B.1.617 India, Delta+ (Vietnamese variant), Epsilon (B.1.429, B.1.427), Omicron B.1.1.529 did affect the effectiveness of the virus detection with the SAVD test. All analyzed 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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Approved for external release by Sabina Żołędowska, CQO
Date of approval: 29.11.2021
Signature: Sabina Żołędowska

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