Gdansk, 11th May 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: ***10.05.2021***

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.

3. Method

Date of the test:	--
Place of the test:	***GeneMe, ul. Szybowcowa 8a, 80-298 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.*
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.*

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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)

The ORF1ab gene fragment targeted for the SAVD assay includes nucleotides 13342-13460. 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*	

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			Gly5530Cys	*G16852T*	
			Met5753Ile	*G17523T*	
			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*	

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		SerGlyPhe 3675-3677 deletion	*11288-11296 deletion*	

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 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).*

1. *Lopez-Rincon, C. A. Perez-Romero, A. Tonda, L. Mendoza-Maldonado, E. Claassen, J. Garssen, A. D. Kraneveld, Design of Specific Primer Sets for the Detection of B.1.1.7, B.1.351 and P.1 SARS-CoV-2 Variants using Deep Learning, bioRxiv January 21, 2021.*
2. *T. Tapp, First Cases of Brazilian And More Contagious South African Covid-19 Variants Detected in Los Angeles; U.K. Variant Surges, April 7, 2021*
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*

A. *M. Voloch et al., Genomic characterization of a novel SARS-CoV-2 lineage from Rio de Janeiro, Brazil, J Virol, 1 March 2021*

4. *N. R. Faria, I. Morales Claro, D. Candido et al., Genomic characterisation of an emergent SARS-CoV-2 lineage in Manaus: preliminary findings, 12 January 2021*
5. *SARS-CoV-2 B.1.617 emergence and sensitivity to vaccine-elicited antibodies, bioRxiv 9 May 2021*
6. *Pragya Dhruv Yadav et al., SARS CoV-2 variant B.1.617.1 is highly pathogenic in hamsters than B.1 variant, bioRxiv 5 May 2021.*
7. *Markus Hoffmann et al., SARS-CoV-2 variant B.1.617 is resistant to Bamlanivimab and evades antibodies induced by infection and vaccination, bioRxiv 5 May 2021.*
8. *Vipul Kumar et al., Possible link between higher transmissibility of B.1.617 and B.1.1.7 variants of SARS-CoV-2 and increased structural stability of its spike protein and hACE2 affinity, bioRxiv 29 April 2021*
9. *Sarah Cherian et al., Convergent evolution of SARS-CoV-2 spike mutations, L452R, E484Q and P681R, in the second wave of COVID-19 in Maharashtra, India, bioRxiv 24 April 2021*
10. *Pragya Yadav et al. Neutralization of variant under investigation B.1.617 with sera of BBV152 vaccinees, bioRxiv 23 April 2021*
11. *https://outbreak.info/situation-reports?pango=B.1.617*
12. *https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/scientific-brief-emerging-variants.html*

Approved for external release by Sabina Żołędowska, CQO
Date of approval: 16.05.2021
Signature: Sabina Żołędowska

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