GENEME	**Research and Development**

NAME: *Marta Skwarecka, Head of RD GeneMe*

DATE: *21.02.2022*

PROPOSED PRODUCT: *ICED*

REVISION: 2.0

1.Title

Detection of different variants of SARS-CoV-2 virus by ICED test.

2. Purpose and scope

The aim of the study is to check the universality of the ICED test for the identification of known variants of the SARS-CoV-2 virus. The most popular variants of the mutant SARS-CoV-2 virus from Alfa to Omicron were analyzed in sillico e.g.: 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: --*** ***Humidity: --***		
The person performing the tests:	***Dr. Marta Skwarecka***		
LOT of reagents analyzed:	**LOT number**	**Trade name**	**Expiry date**
	-	-	-
LOT of reference reagents and trade name:	**LOT number**	**Trade name**	**Expiry date**
	-	-	-

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 ICED test.*
4. *Confirmation or exclusion of the effect of the mutation on the ICED test identification capabilities.*

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4. Tested samples

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

5. Results

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 ICED 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 ICED test.

Virus variant	Country of origin (emergence)	Amino-acid mutation	Nucleotide mutation	Location of mismatch (5'-3') in the ICED test	Detection with the ICED test
Reference Strain: Wuhan-Hu-1, nCoV	*China*	-	-	*absence*	*Yes*
Alpha (B.1.1.7)	*UK*	*T1001I*	*C3267T*	*absence*	*Yes*
		A1708D	*C5388A*	*absence*	
		I2230T	*T6954C*	*absence*	
		SGF 3675-3677 deletion	*11288-11296 deletion*	*absence*	
Beta (B.1.351,S.501Y.V2)	*South Africa*	*Thr265I*	*C1059T*	*absence*	*Yes*
		L1655Asn	*G5230T*	*absence*	
		L3353R	*A10323AG*	*absence*	
Gamma (B.1.1.28.1, P1)	*Brazil*	*synonymous mutation*	*T733C*	*absence*	*Yes*
		synonymous mutation	*C2749T*	*absence*	
		S1188L	*C3828T*	*absence*	
		L1795Q	*A5648C*	*absence*	

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		synonymous mutation	*A6319G*	*absence*	
		synonymous mutation	*A6613G*	*absence*	
		synonymous mutation	*C12778T*	*absence*	
		synonymous mutation	*C13860T*	*absence*	
		E1264N	*G17259T*	*absence*	
		synonymous mutation	*C100T*	*absence*	
		L3468V	*T10667G*	*absence*	
		synonymous mutation	*C11824T*	*absence*	
		L3930F	*C12053T*	*absence*	
Delta (B.1.617.2)	*India*	*P4715L*	*14408-14410*	*absence*	*Yes*
		P5401L	*16466-16468*	*absence*	
		G5063S	*20515-20517*	*absence*	
Delta+ (B.1.617.2+)	*Vietnamese*	*synonymous mutation*	*C3037T*	*absence*	*Yes*
		synonymous mutation	*C3457T*	*absence*	
		T1567I	*C4965T*	*absence*	
		synonymous mutation	*G8491A*	*absence*	
		T3646A	*A11201G*	*absence*	
		P4715L	*C14408T*	*absence*	
		synonymous mutation	*G14772A*	*absence*	

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		synonymous mutation	*C16134T*	*absence*	
		G5530C	*G16852T*	*absence*	
		M5753I	*G17523T*	*absence*	
		L6711R	*A20396G*	*absence*	
		S6713A	*T20401G*	*absence*	
		T1001I	*C3267T*	*absence*	
		A1708D	*C5388A*	*absence*	
		I2230T	*T6954C*	*absence*	
		SGF 3675-3677 deletion	*11287-11295 deletion*	*absence*	
		P4715L	*14407-14409*	*absence*	
		P5401L	*16465-16467*	*absence*	
		G5063S	*15451-15453*	*absence*	
Omicron BA.1 (B.1.1.529)	*South Africa*	*K856R*	*2830-2832*	*absence*	*Yes*
		SL2083-2084I	*6511-6516*	*absence*	
		A2710T	*8392-8394*	*absence*	
		T3255I	*10027-10029*	*absence*	
		P3395H	*10447-10449*	*absence*	
		3674-3676 deletion	*33056-11292*	*absence*	
		I3758V	*11536-11538*	*absence*	
		P4715L	*14407-14409*	*absence*	

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		I5967V	*18163-18165*	*absence*	
Omicron BA.2 (BA.2)	*South Africa*	*S135R*	*667-669*	*absence*	*Yes*
		T842I	*2788-2790*		
		G1307S	*3651-3653*		
		L3027F	*9343-9345*		
		T3090I	*9532-9534*		
		T3255I	*10027-10029*		
		P3395H	*10447-10449*		
		del 3675-3677	*del11287-11295*		
		P4715L	*14407-14409*		
		R5716C	*17410-17412*		
		I5967V	*18163-18165*		
		T6564I	*19954-19956*		
Epsilon (B.1.429, B.1.427)	*California (USA)*	*T265I*	*1057-1059*	*absence*	*Yes*
		S3158T	*9736-9738*	*absence*	
		I4205V	*12877-1279*	*absence*	
		P314L	*1204-1206*	*absence*	
		P976L	*3190-3192*	*absence*	
		D1183T	*3811-3813*	*absence*	
Zeta (B.1.1.28.2, P2)	*Brazil*	-	-	*absence*	*Yes*
Eta	*Worldwide*	*L4715F*	*14407-14409*	*absence*	*Yes*

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(B.1.525)					
Theta (B.1.1.28.3, P3)	*Philippines*	*L3201P*	*9865-9867*	*absence*	*Yes*
		D3681E	*11305-11307*	*absence*	
		L3930F	*12052-12054*	*absence*	
		P4715L	*14407-14409*	*absence*	
Iota (B.1.526)	*USA*	*del3675-3677*	*11287-11295*	*absence*	*Yes*
Kappa (B.1.617.1)	*India*	*synonymous mutation*	*C3037T*	*absence*	*Yes*
		synonymous mutation	*C3457T*	*absence*	
		T1567I	*C4965T*	*absence*	
		synonymous mutation	*G8491A*	*absence*	
		T3646A	*A11201G*	*absence*	
		P4715L	*C14408T*	*absence*	
		synonymous mutation	*G14772A*	*absence*	
		synonymous mutation	*C16134T*	*absence*	
		G5530C	*G16852T*	*absence*	
		M5753I	*G17523T*	*absence*	
		K6711R	*A20396G*	*absence*	
		S6713A	*T20401G*	*absence*	
Lambda (B.1.1.1.C37)	*Peru*	-	-	*absence*	*Yes*

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

6. Conclusions

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The presented analysis shows that none of the mutations occurring in the variants of the SARS-CoV-2 virus, i.e., Alpha, Beta, Gamma, Delta, Omicron, Epsilon, Zeta, Theta, Iota, Kappa and Lambda did affect the effectiveness of the virus detection with the ICED test. All analyzed variants are fully identifiable with the ICED test.

7. References

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*
13. *Jamie Lopez Bernal et al., Effectiveness of COVID-19 vaccines against the B.1.617.2 variant, London 2021*
14. *https://www.france24.com/en/asia-pacific/20210603-who-rules-out-new-hybrid-covid-19-variant-in-vietnam*
15. *https://www.reuters.com/world/asia-pacific/vietnam-detects-hybrid-indian-uk-covid-19-variant-2021-05-29/?utm_medium=Social&utm_source=Twitter*
16. *https://indianexpress.com/article/explained/covid-delta-variant-coronavirus-cases-7344758/*
17. *https://www.republicworld.com/world-news/global-event-news/covid-19-know-about-delta-k417n-mutant-which-has-been-spotted-about-90-times-worldwide.html*
18. *https://outbreak.info/situation-reports?pango=B.1.617.2*
19. *https://outbreak.info/situation-reports?pango=B.1.617*
20. *https://outbreak.info/situation-reports?pango=B.1.1.7*
21. *https://cov-lineages.org/lineage.html?lineage=B.1.427*
22. *https://cov-lineages.org/lineage.html?lineage=B.1.429*
23. *https://viralzone.expasy.org/9556*
24. *Implications of the emergence and spread of the SARS-CoV-2 B.1.1. 529 variant of concern (Omicron) for the EU/EEA, THREAT ASSESSMENT BRIEF, 26.11.2021*
25. *https://cov-lineages.org/lineage.html?lineage=B.1.1.529*
26. *https://viralzone.expasy.org/9556*

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