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Gdańsk, 23. December 2020

To whom it may concern,

On behalf of the GeneMe, taking into account the latest information on the mutation of the S-gene 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 FRANKD RT-LAMP test. This research and development report summarizes our findings regarding the influence of S gene mutations in SARS-CoV-2 on FRANKD primers hybridization. For this purpose, bioinformatic analysis of the mutated sequences of the coronavirus S gene was performed to assess if the point mutation or deletions lay in the hybridization region of FRANKD RT-LAMP primers.

RT-LAMP technologies are less prone to lose specificity and sensitivity in comparison to RT-PCR, because of (i) amplification principle and reaction efficacy (constant amplification, no doubling DNA in each cycle), (ii) more than two primers in the reaction (more targets), (iii) RT-LAMP polymerase features (namely strand display activity of the polymerase).

I can definitively state that FRANKD RT-LAMP assay's ability to detect SARS-CoV-2 remains at the highest level regardless of this new mutation.

GeneMe is working with the head of RT-LAMP in the UK Dr Veronica Fowler and Dr Lesley Scott, the Head of the National Laboratory in South Africa, to ensure a direct line into the latest research from these two countries.

Yours faithfully,

Kasjan Szemiako, Research and Development Director, GeneMe

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GENEME	**Research & Development**

RESEARCH & DEVELOPMENT REPORT	
NAME AND SURNAME	Head of RD Department at GeneMe, Marta Skwarecka, PhD
ANALYZED IVD PRODUCT	FRANKD
DATE OF REPORT PREPARATION	22.12.2020
TITLE OF THE REPORT	The analysis of mutations of S gene in SARS-CoV-2 and their influence on FRANKD primers hybridization

1. Methods

The analysis aimed to show if the recently observed mutations in the SARS-CoV-2 virus genome [1-5] affect the FRANKD test's specificity and assess the potential reduction of efficient hybridization of the 6 primers used in FRANKD.

Bioinformatic analysis of the mutated sequences of the coronavirus S gene was performed, with particular emphasis on nucleotide primers sequences.

The literature review indicated mutations within the S gene: deletion 69-70, deletion 144, N501Y, N439K, D614G, A570D, P681H, T716I, S982A, D1118H (date of accession: 23.12.2020, GeneBank NCBI).

The mutations were analyzed if they lay in the region of FRANKD primers hybridization.

2. Results

Below is presented the nucleotide sequence of the wild-type SARS-CoV-2 S gene with the point mutations marked in red (date of analysis 22 December 2020 – current stage of described mutations in the scientific literature – NCBI GeneBank database). NCBI GeneBank database collects data from all published research papers and GISAID EpiCov database and therefore ensures consistency.

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ATGTTTGTTTTTCTTGTTTTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACCAGAACTCAATT
ACCCCCTGCATACACTAATTCTTTCACACGTGGTGTTTATTACCCTGACAAAGTTTTCAGATCCTCAGTTT
TACATTCAACTCAGGACTTGTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATA**CATGTC**TC
TGGGACCAATGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTCCA
CTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCCAGTCCCTACTT
ATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTTGTAATGATCCATTTTTGGG
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TGCACTTTTGAATATGTCTCTCAGCCTTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTTCAAAAATCT
TAGGGAATTTGTGTTTAAGAATATTGATGGTTATTTTAAAATATATTCTAAGCACACGCCTATTAATTTAG
TGCGTGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTTGCCAATAGGTATTAACATCACT
AGGTTTCAAACTTTACTTGCTTTACATAGAAGTTATTTGACTCCTGGTGATTCTTCTTCAGGTTGGACAGC
TGGTGCTGCAGCTTATTATGTGGGTTATCTTCAACCTAGGACTTTTCTATTAAAATATAATGAAAATGGAA
CCATTACAGATGCTGTAGACTGTGCACTTGACCCTCTCTCAGAAACAAAGTGTACGTTGAAATCCTTCACT
GTAGAAAAAGGAATCTATCAAACTTCTAACTTTAGAGTCCAACCAACAGAATCTATTGTTAGATTTCCTAA
TATTACAAACTTGTGCCCTTTTGGTGAAGTTTTTAACGCCACCAGATTTGCATCTGTTTATGCTTGGAACA
GGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGTCCTATATAATTCCGCATCATTTTCCACTTTTAAG
TGTTATGGAGTGTCTCCTACTAAATTAAATGATCTCTGCTTTACTAATGTCTATGCAGATTCATTTGTAAT
TAGAGGTGATGAAGTCAGACAAATCGCTCCAGGGCAAACTGGAAAGATTGCTGATTATAATTATAAATTAC
CAGATGATTTTACAGGCTGCGTTATAGCTTGGAATTCT**AAC**AATCTTGATTCTAAGGTTGGTGGTAATTAT
AATTACCTGTATAGATTGTTTAGGAAGTCTAATCTCAAACCTTTTGAGAGAGATATTTCAACTGAAATCTA
TCAGGCCGGTAGCACACCTTGTAATGGTGTTGAAGGTTTTAATTGTTACTTTCCTTTACAATCATATGGTT
TCCAACCCACT**AAT**GGTGTTGGTTACCAACCATACAGAGTAGTAGTACTTTCTTTTGAACTTCTACATGC
ACCAGCAACTGTTTGTGGACCTAAAAAGTCTACTAATTTGGTTAAAAACAAATGTGTCAATTTCAACTTCA
ATGGTTTAACAGGCACAGGTGTTCTTACTGAGTCTAACAAAAAGTTTCTGCCTTTCCAACAATTTGGCAGA
GACATT**GCT**GACACTACTGATGCTGTCCGTGATCCACAGACACTTGAGATTCTTGACATTACACCATGTT
CTTTTGGTGGTGTCAGTGTTATAACACCAGGAACAAATACTTCTAACCAGGTTGCTGTTCTTTATCAG**GAT**
GTTAACTGCACAGAAGTCCCTGTTGCTATTCATGCAGATCAACTTACTCCTACTTGGCGTGTTTATTCTAC
AGGTTCTAATGTTTTTCAAACACGTGCAGGCTGTTTAATAGGGGCTGAACATGTCAACAACTCATATGAGT
GTGACATACCCATTGGTGCAGGTATATGCGCTAGTTATCAGACTCAGACTAATTCT**CCT**CGGCGGGCACG
TAGTGTAGCTAGTCAATCCATCATTGCCTACACTATGTCACTTGGTGCAGAAAATTCAGTTGCTTACTCTA
ATAACTCTATTGCCATACCC**ACA**AATTTTACTATTAGTGTTACCACAGAAATTCTACCAGTGTCTATGAC
CAAGACATCAGTAGATTGTACAATGTACATTTGTGGTGATTCAACTGAATGCAGCAATCTTTTGTTGCAAT
ATGGCAGTTTTTGTACACAATTAAACCGTGCTTTAACTGGAATAGCTGTTGAACAAGACAAAAACACCCAA
GAAGTTTTTGCACAAGTCAAACAAATTTACAAAACACCACCAATTAAAGATTTTGGTGGTTTTAATTTTTC
ACAAATATTACCAGATCCATCAAAACCAAGCAAGAGGTCATTTATTGAAGATCTACTTTTCAACAAAGTGA
CACTTGCAGATGCTGGCTTCATCAAACAATATGGTGATTGCCTTGGTGATATTGCTGCTAGAGACCTCATT
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TTCTGCACTGTTAGCGGGTACAATCACTTCTGGTTGGACCTTTGGTGCAGGTGCTGCATTACAAATACCAT
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TTGATTGCCAACCAATTTAATAGTGCTATTGGCAAAATTCAAGACTCACTTTCTTCCACAGCAAGTGCACT
TGGAAAACTTCAAGATGTGGTCAACCAAAATGCACAAGCTTTAAACACGCTTGTTAAACAACTTAGCTCCA
ATTTTGGTGCAATTTCAAGTGTTTTAAATGATATCCTT**TCA**CGTCTTGACAAAGTTGAGGCTGAAGTGCA
AATTGATAGGTTGATCACAGGCAGACTTCAAAGTTTGCAGACATATGTGACTCAACAATTAATTAGAGCTG
CAGAAATCAGAGCTTCTGCTAATCTTGCTGCTACTAAAATGTCAGAGTGTGTACTTGGACAATCAAAAAGA
GTTGATTTTTGTGGAAAGGGCTATCATCTTATGTCCTTCCCTCAGTCAGCACCTCATGGTGTAGTCTTCTT
GCATGTGACTTATGTCCCTGCACAAGAAAAGAACTTCACAACTGCTCCTGCCATTTGTCATGATGGAAAAG
CACACTTTCCTCGTGAAGGTGTCTTTGTTTCAAATGGCACACACTGGTTTGTAACACAAAGGAATTTTTAT
GAACCACAAATCATTACTACA**GAC**AACACATTTGTGTCTGGTAACTGTGATGTTGTAATAGGAATTGTCA
ACAACACAGTTTATGATCCTTTGCAACCTGAATTAGACTCATTCAAGGAGGAGTTAGATAAATATTTTAAG
AATCATACATCACCAGATGTTGATTTAGGTGACATCTCTGGCATTAATGCTTCAGTTGTAAACATTCAAAA
AGAAATTGACCGCCTCAATGAGGTTGCCAAGAATTTAAATGAATCTCTCATCGATCTCCAAGAACTTGGAA
AGTATGAGCAGTATATAAAATGGCCATGGTACATTTGGCTAGGTTTTATAGCTGGCTTGATTGCCATAGTA

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ATGGTGACAATTATGCTTTGCTGTATGACCAGTTGCTGTAGTTGTCTCAAGGGCTGTTGTTCTTGTGGATC
CTGCTGCAAATTTGATGAAGACGACTCTGAGCCAGTGCTCAAAGGAGTCAAATTACATTACACATAA

3. Conclusions

After the analysis of the sequence, GeneMe can state that:

- The observed mutations within the S gene are outside the sequence of primers utilized in the FRANKD test. Sequences of the primers are a trade secret of GeneMe; therefore, they are not disclosed in this report. Upon specific non-disclosure agreements, GeneMe can reveal those sequences to the regulatory bodies – notified bodies.

Further notice:

- It is important to remember that point mutations even in the hybridization region of primer sequences may not significantly influence the efficacy of the primer's hybridization.
- FRANKD, as an RT-LAMP test uses six primers for identification rather than the two normally used by RT-PCR tests.
- FRANKD utilized its own patented Phusion polymerase with the SSB domain that guarantees stronger binding of the primers to the target sequence after the hybridization, limiting the possibility of temperature influence on the dissociation of the primers.
- GeneMe constantly follows scientific information on point mutations in the S gene. Upon any new information, the Head of Research and Development performs *in silico* analysis of the hybridization efficacy of FRANKD primers to the S gene of SARS-CoV-2. In case of a point mutation or a deletion that may influence the test's performance, a new primer set will be designed by changing the point mutated nucleotide for a degenerated nucleotide.
- GeneMe, as a company, manages five diagnostic laboratories in Poland (laboratories certified by the Polish Health Ministry). As a service, GeneMe provides patient sample analysis for the presence of SARS-CoV-2 with the use of RT-PCR and RT-LAMP kits targeting E, Orf1ab, E and S genes. Therefore, GeneMe has the possibility to monitor the

performance of the diagnostic kits available on the market and produced by GeneMe. It was already established that point mutations also occur in other target sequences as presented below [5, 6, 7]:

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

- At GeneMe, we are aware of the possibility of fast mutations that can occur in the group of coronaviruses. Therefore GeneMe has established procedures to monitor those changes and their potential influence on the performance of diagnostic kits produced by us.

- **In our opinion, RT-LAMP technologies are less prone to lose specificity and sensitivity in comparison to RT-PCR, because of (i) amplification principle and reaction efficacy (constant amplification, no doubling DNA in each cycle, (ii) more than two primers in the reaction (more targets), (iii) polymerase features (strand display activity of the polymerase) [8].**

Reference to the literature:

[1] https://www.cogconsortium.uk/news_item/update-on-new-sars-cov-2-variant-and-how-cog-uk-tracks-emerging-mutations/

[2] Thomson EC, Rosen LE, Shepherd JG, et al. The circulating SARS-CoV-2 spike variant N439K maintains fitness while evading antibody-mediated immunity. bioRxiv 2020: 2020.11.04.355842.

[3] Weissman D, Alameh M-G, de Silva T, et al. D614G Spike Mutation Increases SARS CoV-2 Susceptibility to Neutralization. medRxiv 2020: 2020.07.22.20159905.

[4] Rapid increase of a SARS-CoV-2 variant with multiple spike protein mutations observed in the United Kingdom

[5] Preliminary genomic characterization of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations

[6] Khailany RA, Safdar M, Ozaslan M. Genomic characterization of a novel SARS-CoV-2. Gene Rep. 2020;19:100682. doi:10.1016/j.genrep.2020.100682

[7] A Recurrent Mutation at Position 26340 of SARS-CoV-2 Is Associated with Failure of the E Gene Quantitative Reverse Transcription-PCR Utilized in a Commercial Dual-Target Diagnostic Assay

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Journal of Clinical Microbiology Sep 2020, 58 (10) e01598-20; DOI: 10.1128/JCM.01598-20

[8] Olszewski M, Balsewicz J, Nowak M, Maciejewska N, Cyranka-Czaja A, Zalewska-Piątek B, et al. (2015) Characterization of a Single-Stranded DNA-Binding-Like Protein from *Nanoarchaeum equitans*—A Nucleic Acid Binding Protein with Broad Substrate Specificity. PLoS ONE 10(5): e0126563. https://doi.org/10.1371/journal.pone.0126563

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