BioFire® COVID-19 Test SARS-CoV-2 Reactivity

TECHNICAL
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Introduction

The BioFire® COVID-19 Test is a qualitative PCR test for use on FilmArray® 2.0 and FilmArray® Torch systems for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in nasopharyngeal swabs (NPS) eluted in transport medium. Internal controls are used to monitor all stages of the test process. The BioFire COVID-19 Test consists of three independent and non-overlapping assays targeting two SARS-CoV-2 open reading frame sequences: ORF1ab and ORF8. The assays are designed to detect SARS-CoV-2 specifically. Detection of SARS-CoV-2 RNA is based on the combined results of the three assays, any two assay positives result in a "Detected" call, whereas a single assay positive results in an "Equivocal" call.

NOTE: *BioFire tests do not report cycle threshold (Ct) values and the BioFire COVID-19 Test SARS-CoV-2 assays are not intended to monitor for novel mutations.*

Emerging SARS-CoV-2 Variants

Several emerging SARS-CoV-2 variants in the United Kingdom[1] (SARS-CoV-2 VUI 202012/01, lineage B.1.1.7), South Africa[2] (501Y.V2, lineage B.1.351) and Nigeria (P681H, lineage B.1.1.207) have been identified as of potential concern due to reported higher infectivity and transmission rates. To address these variants and other clinically relevant strains, BioFire Defense has performed a comprehensive *in silico* inclusivity analysis of all GISAID EpiCoV complete, high coverage sequences submitted as of January 4th, 2021 and collected after September 1st, 2020 (total of 84,483). These sequences were monitored to identify variant specific mismatches that co-occur in more than one assay, posing a higher risk of false negatives than mismatches in single assays. Submissions with collection dates before September 1st, 2020 were excluded as they were unlikely to represent currently circulating strains. Complete, human-host, high coverage sequences were downloaded from the GISAID database, and sequences containing greater than one percent ambiguities relative to the total genomic length were subsequently removed. **Table 1** reports the number of recent submissions with mismatches within 10bp of the 3' end of the primer for accessions with variation co-occurring on multiple assays.

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Table 1. High-Risk Variant Summary for Recent Submissions to Evaluate Emerging Strains

Collection Date	Assay Target	Assay Target			
		Mismatch Frequencies per Assay (ANY primer per assay)			
		SARS-CoV-2a	SARS-CoV-2d	SARS-CoV-2e	All assays
Sept - Dec 2020	SARS-CoV-2a	708	3	13	0
	SARS-CoV-2d		523	10	
	SARS-CoV-2e			3,450	
	Total # sequences	84,483			

All values represent the number of sequences that contain mismatches within 10bp of the 3' end of any primer in that assay **Light grey shaded** cells are >1%, but <5% of the total for the time interval.

BioFire Defense compared these co-occurring, high-risk sequences to sequences identified in the GISAID database as belonging to the emerging UK and South Africa lineages. From the two novel lineages, only three sequences (with identical genomes across non-ambiguous regions) contained variants to more than one assay. These sequences were isolated from the Netherlands in December 2020 and belong to the UK B.1.1.7 lineage. Because the mismatch is located near the 5' end of the SARS-CoV-2e assay primer, the risk of a false negative is low in these sequences. Based on our current monitoring, no high-frequency mutations in the emerging UK, South African and Nigerian lineages would be predicted to impact the detection of SARS-CoV-2 by the BioFire® COVID-19 Test.

Certain mutations are shared across the UK, South African and Nigerian lineages. Newly emerging variants may contain one or more of these mutations, as well as other, potentially novel genomic variations. **Table 2** shows the individual amino acid changes that characterize the UK, South African and Nigerian lineages, their respective lineage, and the effects of each mutation on individual assays and the expected overall detection result of the BioFire® COVID-19 Test. When a mutation was co-located in a gene targeted by the BioFire COVID-19 Test, the primer site regions were analyzed for risk of false negatives. If the mutation was located in a gene not targeted by the BioFire® COVID-19 Test, the assay was reported as not targeted. Based on these analyses, none of the mutations defining the emerging strains in the UK (VOC202012/01), South Africa (501Y.V2) and Nigeria (P681H) pose a risk to the BioFire COVID-19 Test detection performance.

Table 2. List of Mutations Identified for Emerging Strains and Effect on BioFire COVID-19 Test Assay Reactivity

Mutation	SARS-Cov-2 Lineage/Variant			COVID-19 Test Assays			Estimated BioFire COVID-19 Test Overall Detection Result*
Amino Acid	UK B1.1.7 VOC 202012/01	South Africa B.1.351 501Y.V2	Nigeria B1.1.207 P681H	SARS-CoV-2a (ORF1ab)	SARS-CoV-2d (ORF1ab)	SARS-CoV-2e (ORF8a)	
ORF1ab							
T1001I	X			Unaffected	Unaffected	Not targeted	Unaffected
A1708D	X			Unaffected	Unaffected		Unaffected
I2230T	X			Unaffected	Unaffected		Unaffected
Δ3675-7 SGF	X			Unaffected	Unaffected		Unaffected
T265I		X		Unaffected	Unaffected		Unaffected
K1655N		X		Unaffected	Unaffected		Unaffected
H2799Y		X		Unaffected	Unaffected		Unaffected
S2900L		X		Unaffected	Unaffected		Unaffected
K3353R		X		Unaffected	Unaffected		Unaffected
D4527Y		X		Unaffected	Unaffected		Unaffected
P4715L		X		Unaffected	Unaffected		Unaffected
T5912I		X		Unaffected	Unaffected		Unaffected
ORF8							
Q27 STOP	X			Not targeted	Not targeted	Unaffected	Unaffected
R52I	X					Unaffected	Unaffected
Y73C	X					Located in 5' end of one primer; no effect on performance expected.	Unaffected
Spike (S)							
Δ69-70	X			Not targeted	Not targeted	Not targeted	Unaffected
Δ144	X						Unaffected
N501Y	X	X					Unaffected
A570D	X						Unaffected
P681H	X		X				Unaffected
T716I	X						Unaffected
S982A	X						Unaffected
D1118H	X						Unaffected
K417N		X					Unaffected
E484K		X					Unaffected
D614G		X					Unaffected
L18F		X					Unaffected
D80A		X					Unaffected
D215G		X					Unaffected
R246I		X					Unaffected
A701V		X					Unaffected
Nucleocapsid (N)							
D3L	X			Not targeted	Not targeted	Not targeted	Unaffected
S235F	X						Unaffected
T205I		X					Unaffected
Envelope (E)							
P71L		X		Not targeted	Not targeted	Not targeted	Unaffected
ORF3a							
Q57H		X		Not targeted	Not targeted	Not targeted	Unaffected
S171L		X					Unaffected

*Any two assays positive.

In summary, these analyses show that the risk of false negatives with the BioFire® COVID-19 Test due to the SARS-COV-2 United Kingdom (SARS-CoV-2 VUI 202012/01, lineage B.1.1.7), South African (501Y.V2, lineage B.1.351) and Nigerian (P681H, lineage B.1.1.207) variant lineages is low.

Global *in silico* SARS-CoV-2 Variant Analysis

An initial evaluation of SARS-CoV-2 unique genomes was performed in March 2020, as stated in the current COVID-19 Test IFU. BioFire has performed periodic updates of this *in silico* analysis with the most recent performed on January 4th, 2021. Based on the most recent analyses (Sept - Dec 2020 collection date; submitted prior to Jan 4th, 2021) all variants would be detected by the BioFire COVID-19 Test.

Conclusions

1. The detection of SARS-CoV-2 by the BioFire® COVID-19 Test assays is not affected by the circulating UK variants reported in the Genomics UK Consortium report[1], or by the South African[2] and Nigerian variants.

2. Global *in silico* analysis (as of January 4th, 2021) predicts that the BioFire® COVID-19 Test SARS-CoV-2 assays will detect all 84,484 sequences evaluated.

Bioinformatics for SARS-CoV-2 is expanding at a rapid rate since the first confirmed incidence of human infection in late 2019. Tens of thousands of viral whole genome sequences are being evaluated and submitted to public and private databases on a monthly basis. As the pandemic persists and viral genomes evolve, monitoring of assay reactivity with new sequences is important for understanding the performance of the SARS-CoV-2 assays in the BioFire® COVID-19 Test. BioFire Defense is committed to monitoring these new sequences and will perform regular revised *in silico* analyses of the BioFire COVID-19 Test SARS-CoV-2 assays.

References

1. COG-UK update on SARS-CoV-2 Spike mutations of special interest – Report 1 Dec 20, 2020

2. Tegally *et al*. 2020 Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. medRxiv, https://doi.org/10.1101/2020.12.21.20248640: (2020)

Technical Support Contact Information

BioFire Defense is dedicated to providing the best customer support available. If you have any questions or concerns about this process, please contact the FilmArray Technical Support team for assistance.

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