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

Introduction

The BioFire® COVID-19 Test is a multiplexed, nested reverse transcription (RT)-PCR test designed for use with BioFire® FilmArray® system for the qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in respiratory and saliva specimens. The BioFire COVID-19 Test consists of seven assays targeting four SARS-CoV-2 open reading frames: ORF1ab, S gene, ORF8, and N gene. The assays are designed to detect SARS-CoV-2 specifically. If any of the seven assays are 'Detected', the overall test result for SARS-CoV-2 will be 'Detected'.

As of June 19, 2022, BioFire Defense **predicts that existing and emerging SARS-CoV-2 variants have no impact to BioFire COVID-19 Test performance.**

Global in silico SARS-CoV-2 Inclusivity Monitoring

Emerging SARS-CoV-2 variants can harbor clinical phenotypes affecting vaccine efficacy, virulence, and transmissibility. Because such strains pose an increased threat to public health, the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) classifies such emerging lineages as Variants of Concern (VOCs), Variants of Interest (VOIs), Variants Under Monitoring (VUM), and VOC-Lineages Under Monitoring (VOC-LUM) (**Table 1**). Lineages under monitoring are currently a subset of the lineages within the VOC variant (VOC-LUM). The variant frequencies in the most recent 3-month and 1-month intervals are shown in **Table 1**.

Table 1. Variants of Concern (VOC) and Lineages Under Monitoring (LUM)

	Pangolin Lineage	WHO Variant Designation	April - June 2022 # (%)	June 2022 # (%)
Variant of Concern-Lineages Under Monitoring (VOC-LUM)	BA.2.12.1	Omicron	15,979 (11.94%)	1,699 (37.53%)
	BA.2.13		905 (0.68%)	41 (0.91%)
	BA.2.9.1		319 (0.24%)	8 (0.18%)
	BA.2.11		41 (0.03%)	2 (0.04%)
	BA.4		56 (0.04%)	21 (0.46%)
	BA.5		317 (0.24%)	74 (1.63%)
Variant of Concern (VOC)	Recombinants[1]		69 (0.05%)	2 (0.04%)
	Other Omicron		115,945 (86.6%)	2,671 (59.00%)
Other			252 (0.19%)	9 (0.20%)
Total			**133,883 (100%)**	**4,527 (100%)**

[1]All recombinant lineages of Omicron sublineages (e.g., XE is a recombinant of BA.1 and BA.2).
Note: Variants designated by the WHO on June 19th, 2022. There are currently no VOIs or VUMs designated.

BioFire Defense performs regular in silico monitoring to identify SARS-CoV-2 viruses predicted to have reduced reactivity for the BioFire COVID-19 Test. These analyses consider currently recognized VOCs, VOC-LUMs, VOIs and VUMs as well as yet-to-be classified lineages gaining prominence globally. To perform these analyses, SARS-CoV-2 whole genome sequences deposited in the GISAID EpiCoV™ database are evaluated from recently collected patient samples. These sequences capture strains likely to be circulating and offer the best picture of how SARS-CoV-2 is evolving through human transmission. A total of 169,366 GISAID sequences collected from April 1, 2022, through June 19, 2022, were analyzed for this report.

The BioFire COVID-19 Test consists of seven assays targeting different regions of the viral genome. The number of assays reduces the risk of false negatives from emerging variants as co-occurring mutations affecting the primer binding regions of multiple assays are rare. Sequences with mutations falling within 10 base pairs (bp) from the 3' end of the primer binding region are considered a greater risk to reactivity as they may result in reduced amplification efficiency. Sequences meeting both conditions (i.e., co-occurring mutations to the 3' end of primers in multiple assays) are summarized in **Table 2**. These sequences are broken out by their VOC status to illustrate predicted reactivity specific to each variant.

Table 2. Summary of Higher Risk Co-occurring Mutations in Sequences Collected from April 1, 2022, to June 19, 2022

Assays Affected	Frequency of Sequences with High-Risk Mutations[1] (%)						
	Variants of Concern (VOCs): Omicron					Other	All Sequences
	VOC-LUM			X[3]	Other Omicron[4]		
	BA.2 (grouped)[2]	BA.4	BA.5				
2a	0.77	-	-	-	0.40	1.59	0.45
2c	0.29	-	2.21	-	0.93	3.17	0.85
2d	0.30	-	2.21	-	0.88	3.17	0.81
2e	0.70	1.79	1.89	-	0.79	1.19	0.78
2f	0.84	1.79	1.89	--	1.64	3.97	1.54
2g	99.85	100	100	100	99.81	92.46	99.80
2h	0.38	-	-	-	0.57	0.79	0.55
Frequency of Sequences with Co-occurring Mutations (%)							
Any 3	0.92	1.79	3.79	-	1.38	4.76	1.34
Any 4	-	-	-	-	0.05	-	0.05
Any 5	-	-	-	-	0.00	-	0.00
Any 6	-	-	-	-	-	-	-
All 7	-	-	-	-	-	-	-
Total sequences # %	17,244 12.88	56 0.04	317 0.24	69 0.05	115,945 86.60	252 0.19	133,883 100.00

[1]Non-ambiguous mutations under the primer binding regions that fall within 10bp of 3' end of the primer were considered in this analysis.
[2]VOC-LUMs that are sublineages of BA.2 are grouped. The lineage composition is shown in Table 1.
[3]Includes all recombinant lineages of Omicron sublineages (e.g., XE is a recombinant of BA.1 and BA.2).
[4]The majority are classified as BA.2 or other BA.2 sublineages.
Note: Assay names are abbreviated as SARS-COV-2a (2a), SARS-COV-2d (2d), etc.
Note: Dashes (-) indicate that no sequences in the dataset meet these criteria.

As summarized in **Table 2,** nearly all sequences (133,879/133,883) have at least three assays unaffected by high-risk mutations and approximately 99% of sequences have at least five assays unaffected by high-risk mutations. No sequences carry high risk mutations to six or all seven assays. Therefore, there is a low risk of false negatives for the BioFire COVID-19 Test among currently circulating strains.

Conclusion

Global in silico analyses of circulating SARS-CoV-2 sequences (Through June 19, 2022) predict that the BioFire® COVID-19 Test performance is unaffected and continues to have reliable detection of VOCs, VOC-LUMs, VOIs, and VUMs defined by the CDC and WHO.

Continuous SARS-CoV-2 Variant Monitoring

The amount of genetic information for SARS-CoV-2 is expanding at a rapid rate since the first confirmed incidence of human infection in late 2019. Thousands of viral whole genome sequences are being evaluated and submitted to public databases monthly. As the pandemic persists and viral genomes evolve, monitoring of assay reactivity with new sequences is critical for understanding the performance of the SARS-CoV-2 assays in the BioFire COVID-19 Test. BioFire Defense is continuously monitoring these new sequences and will perform regular revised in silico analyses of the BioFire COVID-19 Test SARS-CoV-2 assays.

Technical Support Contact Information

BioFire Defense is dedicated to providing the best customer support available. For any questions or concerns, please contact the BioFire Technical Support team for assistance.

General Information
Email: support@biofiredefense.com
Phone: 1-801-262-3592
Fax: 1-801-447-6907