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

TECHNICAL
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Introduction

The BioFire® COVID-19 Test is a multiplexed, nested reverse transcription (RT)-PCR test designed for use with the 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 September 13, 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 World Health Organization (WHO) classifies such lineages as Variants of Concern (VOCs) and Subvariants Under Monitoring (SUM) as of September 13, 2022 (see https://www.who.int/activities/tracking-SARS-CoV-2-variants for details). Currently, the Omicron variant is the only VOC, and it consists of several subvariants classified as SUM by the WHO. The variant frequencies in the most recent 3-month and 1-month intervals are shown in **Table 1**.

Table 1. Subvariants Under Monitoring (SUM) and Variants of Concern (VOC)

Pangolin Lineage	WHO Variant Designation	June - August 2022 # (%)	August 2022 # (%)
Subvariants Under Monitoring (SUM)			
BA.2.12.1	Omicron	15,957 (25.06)	269 (3.34)
BA.2.75	Omicron	1,268 (1.99)	591 (7.34)
BA.4	Omicron	1,345 (2.11)	227 (2.82)
BA.5	Omicron	15,949 (25.04)	5,385 (66.9)
Variants of Concern (VOC)			
Recombinant[1]	Omicron	9 (0.01)	0
Non SUM	Omicron	25,764 (40.45)	465 (5.78)
Lineages Not Currently Under Monitoring by the WHO			
		3,394 (5.33)	1,112 (13.82)
All Sequences			
		63,686 (100)	**8,049 (100)**

[1] *All Recombinant lineages of Omicron sublineages (e.g., XE is a recombinant of BA.1 and BA.2)*

Note: Yellow: ≥5% change between periods

Note: Variants designated by the WHO on September 13th, 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 and SUMs 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 63,686 GISAID sequences submitted before September 13, 2022, with collection dates from June 1, 2022 through August 31, 2022 were analyzed for this report.

The BioFire COVID-19 Test consists of seven assays targeting different regions of the viral genome. The use of seven 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 SUM status to illustrate predicted reactivity specific to each subvariant.

Table 2. Summary of Higher Risk Co-occurring Mutations in Sequences Collected from June 1, 2022 to August 31, 2022

Frequencies of Sequences with High-Risk Mutations[1]										
	Mutated Sequences by Assay							Co-occurring Mutated Sequences[2]		
Pangolin Lineage	2a	2c	2d	2e	2f	2g	2h	5	≥6	Sequences by Lineage
Subvariants Under Monitoring (SUM)										
BA.2.12.1	202 (1%)	127 (1%)	115 (1%)	136 (1%)	169 (1%)	15,946 (100%)	99 (1%)	0	0	15,957 (25.1%)
BA.2.75	11 (1%)	4 (0%)	4 (0%)	12 (1%)	24 (2%)	1,266 (100%)	3 (0%)	1 (0%)	0	1,268 (2%)
BA.4	4 (0%)	4 (0%)	4 (0%)	17 (1%)	22 (2%)	1,343 (100%)	2 (0%)	1 (0%)	0	1,345 (2.1%)
BA.5	79 (0%)	137 (1%)	142 (1%)	193 (1%)	342 (2%)	15,892 (100%)	64 (0%)	1 (0%)	0	15,949 (25%)
Variants of Concern (VOC)										
Recombinant[3]	0	0	0	0	0	9 (100%)	0	0	0	9 (0%)
Non SUM	138 (1%)	283 (1%)	292 (1%)	235 (1%)	620 (2%)	25,699 (100%)	283 (1%)	1 (0%)	0	25,764 (40.5%)
Lineages Not Currently Under Monitoring by the WHO										
	7 (0%)	22 (1%)	27 (1%)	27 (1%)	38 (1%)	3,351 (99%)	10 (0%)	0	0	3,394 (5.3%)
Summary: All Sequences by Assays										
	441 (0.7%)	**577 (0.9%)**	**584 (0.9%)**	**620 (1%)**	**1,215 (1.9%)**	**63,506 (99.7%)**	**461 (0.7%)**	**4 (0%)**	**0**	**63,686 (100%)**

[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] Mutated sequences co-occurring on multiple assays, by # assays
[3] All Recombinant lineages of Omicron sublineages (e.g., XE is a recombinant of BA.1 and BA.2)
Color guide - Yellow: mutations occur at ≥5%; Blue: mutations occur at <1%
Note: Assay names are abbreviated as SARS-COV-2a (2a), SARS-COV-2d (2d), etc.

As summarized in **Table 2,** nearly all sequences (63,682/63,686) have at least three 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 August 31, 2022) predict that the BioFire® COVID-19 Test performance is unaffected and continues to have reliable detection of SARS-COV-2 sequences including all SUMs defined by the 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
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Conditions of Authorization

The BioFire COVID-19 Test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

The BioFire COVID-19 Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of the BioFire COVID-19 Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.