

# 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 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 October 10, 2023, 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 (VOC), Variants of Interest (VOI), and Variants Under Monitoring (VUM) as of October 10, 2023 (see <https://www.who.int/activities/tracking-SARS-CoV-2-variants> for details). Currently, there are no VOCs. The variant frequencies in the most recent 3-month and 1-month intervals are shown in **Table 1**.

**Table 1. Variants of Interest (VOI) and Variants Under Monitoring (VUM)**

Pangolin Lineage	July - September 2023 # (%)	September 2023 # (%)
Variants of Interest (VOI)		
XBB.1.5	350 (6.96%)	56 (3.43%)
XBB.1.16	939 (18.67%)	345 (21.1%)
EG.5	1,037 (20.62%)	419 (25.63%)
Variants Under Monitoring (VUM)		
BA.2.86	18 (0.36%)	16 (0.98%)
CH.1.1	45 (0.89%)	1 (0.06%)
XBB	1 (0.02%)	0
XBB.1.9.1	69 (1.37%)	17 (1.04%)
XBB.1.9.2	41 (0.82%)	4 (0.24%)
XBB.2.3	252 (5.01%)	64 (3.91%)
Variants Not Currently Under Monitoring by the WHO		
Omicron	1,662 (33.04%)	522 (31.93%)
Non-Omicron	590 (11.73%)	191 (11.68%)
Unassigned	26 (0.52%)	0
<b>All Sequences</b>		
	<b>5,030 (100%)</b>	<b>1,635 (100%)</b>

Note: Yellow: ≥5% change between periods

Note: Variants designated by the WHO as of October 10th, 2023. There are currently no VOCs designated.

## TECHNICAL NOTE

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, 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 5,030 GISAID sequences submitted before October 10, 2023, with collection dates from July 1, 2023, through September 30, 2023, 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 VOI and VUM status to illustrate predicted reactivity specific to each subvariant.

**Table 2. Summary of Higher Risk Co-occurring Mutations in Sequences Collected from July 1, 2023 to September 30, 2023**

Pangolin Lineage	Mutated Sequences by Assay							Co-occurring Mutated Sequences <sup>2</sup>		Sequences by Lineage
	2a	2c	2d	2e	2f	2g	2h	5	≥6	
	<b>Frequencies of Sequences with High-Risk Mutations<sup>1</sup></b>									
<b>Variants of Interest (VOI)</b>										
XBB.1.5	15 (4%)	9 (3%)	11 (3%)	13 (4%)	44 (13%)	350 (100%)	6 (2%)	0	0	350 (7%)
XBB.1.16	2 (0%)	13 (1%)	14 (1%)	40 (4%)	41 (4%)	939 (100%)	5 (1%)	0	0	939 (18.7%)
EG.5	5 (0%)	14 (1%)	15 (1%)	6 (1%)	9 (1%)	1,037 (100%)	6 (1%)	0	0	1,037 (20.6%)
<b>Variants Under Monitoring (VUM)</b>										
BA.2.86	0	0	0	0	0	18 (100%)	1 (6%)	0	0	18 (0.4%)
CH.1.1	0	2 (4%)	2 (4%)	5 (11%)	3 (7%)	45 (100%)	0	0	0	45 (0.9%)
XBB	0	0	0	0	0	1 (100%)	0	0	0	1 (0%)
XBB.1.9.1	0	0	0	2 (3%)	2 (3%)	69 (100%)	0	0	0	69 (1.4%)
XBB.1.9.2	0	0	0	1 (2%)	1 (2%)	41 (100%)	0	0	0	41 (0.8%)
XBB.2.3	6 (2%)	1 (0%)	1 (0%)	2 (1%)	4 (2%)	252 (100%)	1 (0%)	0	0	252 (5%)
<b>Variants Not Currently Under Monitoring by the WHO</b>										
Omicron	128 (8%)	14 (1%)	17 (1%)	25 (2%)	47 (3%)	1,661 (100%)	12 (1%)	0	0	1,662 (33%)
Non-Omicron	0	3 (1%)	2 (0%)	1 (0%)	3 (1%)	590 (100%)	7 (1%)	0	0	590 (11.7%)
Unassigned	0	0	0	0	0	25 (96%)	0	0	0	26 (0.5%)
<b>Summary: All Sequences by Assays</b>										
	156 (3.1%)	56 (1.1%)	62 (1.2%)	95 (1.9%)	154 (3.1%)	5,028 (100%)	38 (0.8%)	0	0	5,030 (100%)

<sup>1</sup> Non-ambiguous mutations under the primer binding regions that fall within 10bp of 3' end of the primer were considered in this analysis.

<sup>2</sup> Mutated sequences co-occurring on multiple assays, by # assays

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**, all sequences (5,030/5,030) have at least three assays unaffected by high-risk mutations. No sequences carry high risk mutations to five, 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 September 30, 2023) predict that the BioFire COVID-19 Test performance is unaffected and continues to have reliable detection of SARS-COV-2 sequences including all VOIs and VUMs 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](mailto:support@biofiredefense.com)

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## TECHNICAL NOTE

### 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.