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

TECHNICAL NOTE

Introduction

The BioFire® COVID-19 Test is a multiplexed, nested reverse transcription (RT)-PCR test designed for use with BioFire® FilmArray® systems 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 three independent and non-overlapping assays targeting two SARS-CoV-2 open reading frames: ORF1ab and ORF8. The assays are designed to detect SARS-CoV-2 specifically.

As of May 16, 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), and Variants Under Monitoring (VUM) (**Table 1).** The variant frequencies in a 3-month and 1-month interval are shown in **Table 1**.

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

	Pangolin Lineage	WHO Designation	Location First Identified	March - May 2022 # (%)	May 2022 # (%)
Variants of Concern	B.1.617.2	Delta	India	238 (0.1%)	1 (0.0%)
	BA.1	Omicron	South Africa	1,981 (1.2%)	3 (0.1%)
	BA.2			165,113 (98.3%)	4,461 (99.8%)
	Recombinants[1]			104 (0.1%)	5 (0.1%)
	Other			184 (0.1%)	1 (0.0%)
Variants Under Monitoring	XD[2]	Omicron/ Delta	France/ Denmark	-	-
	B.1.640	n/a	Africa/Europe	-	-
Other				362 (0.2%)	1 (0.0%)
Total				167,982 (100%)	4,472 (100%)

[1]All recombinant lineages of Omicron sublineages (e.g., XE is a recombinant of BA.1 and BA.2).
[2]XD is a recombinant lineage of Omicron BA.1 and Delta.
Note: Variants are designated by the WHO as of May 16, 2022. There are currently no VOIs designated.

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DFA2-PRT-0129-05

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 currently circulating and offer the best picture of how SARS-CoV-2 is evolving through human transmission. A total of 167,982 GISAID sequences collected from March 1, 2022 up to May 16, 2022, were analyzed for this report.

The BioFire COVID-19 Test consists of assays targeting different regions of the viral genome. This test design reduces the risk of new variants going undetected (false negatives), because test performance would only be impacted if mutations occurred on multiple assays at once. 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 March 1, 2022 to May 16, 2022

Assays Affected[2]	Number of Sequences Containing Mutations within 10 bp of the 3' End of the Primer Across Multiple Assays (%)							
	Variants of Concern (VOCs)					VUM	Other	All Sequences
	Delta	Omicron						
		BA.1	BA.2	X[1]	Other			
2a & 2d	1 0.42%	-	-	-	-	-	-	1 0.00%
2d & 2e	-	-	-	-	-	-	-	-
2a & 2e	-	-	2 0.00%	-	-	-	-	2 0.00%
All Assays	-	-	-	-	-	-	-	-
Total # Sequences (%)	**238 0.1%**	**1,981 1.2%**	**165,113 98.3%**	**104 0.1%**	**184 0.1%**	**0 0.0%**	**362 0.2%**	**167,982 100%**

[1]Includes all recombinant lineages of Omicron sublineages (e.g., XE is a recombinant of BA.1 and BA.2).
[2]Assay names are abbreviated as SARS-COV-2a (2a), SARS-COV-2d (2d), and SARS-CoV-2e (2e).
Additional notes: Dashes (-) indicate that no sequences in the dataset meet the specified criteria. Only non-ambiguous mutations under the primer binding regions that fall within 10bp of 3' end of the primer were considered in this analysis.

As summarized in **Table 2,** less than 0.002% of sequences (3/167,982) carry high risk mutations to more than one assay, and no sequences carry high risk mutations to all three 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 (up to May 16, 2022) predict that the BioFire® COVID-19 Test performance is unaffected and continues to have reliable detection of VOCs, 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. Hundreds of 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.

Technical Support Team
Email: 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.