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 February 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 Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) classifies such emerging lineages as Variants of Concern (VOCs) and Variants of Interest (VOIs) (**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 of Interest (VOI)

	Pangolin lineage	WHO designation	Location first identified	Nov 2021 through Jan 2022 # (%)	Jan 2022 # (%)
Variants of Concern	B.1.1.7	Alpha	United Kingdom	150 (0.0%)	1 (0.0%)
	B.1.351	Beta	South Africa	9 (0.0%)	-
	P.1	Gamma	Brazil	152 (0.0%)	2 (0.0%)
	B.1.617.2	Delta	India	937,652 (96.7%)	22,512 (40.5%)
	B.1.1.529	Omicron	South Africa	37,231 (3.8%)	32,630 (58.8%)
Variants of Interest	B.1.621	Mu	Columbia	37 (0.0%)	-
	C.37	Lambda	Peru	22 (0.0%)	-
Other				4,352 (0.4%)	389 (0.7%)
Total				979,546 (100%)	55,534 (100%)

Variants of Concern and Interest designated by the WHO on February 17, 2022.

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 VOIs 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 979,546 GISAID sequences collected from November 2021 through January 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 because a virus would have to present with a collection of mutations that result in multiple assay failure, to result in a missed detection (false negative). Sequences with mutations falling within 10 base pairs (bp) of the 3' end of the primer binding region are considered a greater risk to reactivity as they are more likely to disrupt the PCR reaction. 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 November 2021 to January 2022

Assays affected		Number of sequences containing mutations within 10 bp of the 3' end of the primer across multiple assays (%)						
	Variants of Concern (VOCs)					Other[1]	All sequences
	Alpha	Beta	Gamma	Delta	Omicron		
2a & 2d	-	-	-	341 (0.04%)	-	-	341 (0.04%)
2d & 2e	-	-	-	394 (0.04%)	-	-	394 (0.04%)
2a & 2e	-	-	-	218 (0.02%)	-	1 (0.02%)	219 (0.02%)
All assays	-	-	-	5 (0.00%)	-	-	5 (0.00%)
Total # sequences (%)	**150 (0.0%)**	**9 (0.0%)**	**152 (0.0%)**	**937,652 (95.7%)**	**37,231 (3.8%)**	**4,352 (0.4%)**	**979,546 (100%)**

Assay names are abbreviated as SARS-COV-2a (2a), SARS-COV-2d (2d), and SARS-CoV-2e (2e).
Dashes (-) indicate that no sequences in the dataset meet these criteria.
Non-ambiguous mutations under the primer binding regions that fall within 10bp of 3' end of the primer were considered in this analysis.
[1]Includes VOIs.

As summarized in **Table 2,** fewer than 0.1% of the sequences carry high risk mutations to more than one assay, and only five 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 February 13, 2022) predict that the BioFire® COVID-19 Test performance is unaffected and continues to have reliable detection of VOCs and VOIs defined by the CDC and WHO.

Continuous SARS-CoV-2 Variant Monitoring

Bioinformatics 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 and private databases monthly. 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 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

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.