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

TECHNICAL
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Introduction

BioFire Defense predicts that existing and emerging SARS-CoV-2 variants have no impact to BioFire COVID-19 Test performance, including the recently identified Omicron variant (B.1.1.529).

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.

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).**

Table 1. Variants of Concern (VOC) and Variants of Interest (VOI)

	Pangolin lineage	CDC and WHO designation	Location first identified
Variants of Concern	B.1.1.7	Alpha	United Kingdom
	B.1.351	Beta	South Africa
	P.1	Gamma	Brazil
	B.1.617.2	Delta	India
Variants of Interest	B.1.621	Mu	Columbia
	C.37	Lambda	Peru

Variants of Concern and Variants of Interest designated by the WHO on Nov. 1, 2021

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

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transmission. A total of 1,254,527 GISAID sequences collected from August through October 2021 were analyzed for this report.

The BioFire COVID-19 Test consists of assays targeting different regions of the viral genome. Because a virus would have to present with a collection of mutations that result in multiple assay failures, this test design reduces the risk of new variants going undetected. 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/VOI status to illustrate predicted reactivity specific to each variant.

Table 2. Summary of Higher Risk Co-occurring Mutations in Sequences Collected from August to October 2021

Assays affected	Number of sequences containing mutations within 10 bp of the 3' end of the primer across multiple assays (%)							
	Variants of Concern (VOCs)				Variants of Interest (VOIs)		Other	All sequences
	Alpha	Beta	Gamma	Delta	Mu	Lambda		
2a & 2d	-	-	-	408 (0.03%)	-	-	1 (0.01%)	409 (0.03%)
2d & 2e	1 (0.02%)	-	2 (0.03%)	351 (0.03%)	1 (0.07%)	-	1 (0.01%)	356 (0.03%)
2a & 2e	-	-	-	253 (0.02%)	-	-	2 (0.02%)	255 (0.02%)
All assays	-	-	-	-	-	-	-	-
Total # sequences (%)	**6,033 (0.48%)**	**248 (0.02%)**	**6,109 (0.49%)**	**1,227,528 (97.85%)**	**1,455 (0.12%)**	**125 (0.01%)**	**13,029 (1.04%)**	**1,254,527 (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.

As summarized in **Table 2,** fewer than 0.1% of the sequences carry high risk mutations to more than one assay, and none 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.

Omicron variant analysis

BioFire Defense has performed a separate in silico analysis using all available sequences of the newly emerged Omicron variant/B.1.1.529 (n=206 as of November 30, 2021). The analysis concludes that all Omicron variants will be detected by the BioFire COVID-19 Test with no predicted impact to test performance.

Conclusion

Global in silico analyses of circulating SARS-CoV-2 sequences (up to November 1, 2021, and Omicron sequences as of November 30, 2021) 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
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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.