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

TECHNICAL NOTE

## Introduction

The BioFire® COVID-19 Test is a multiplexed, nested qualitative PCR test designed to use on BioFire® FilmArray® systems for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in respiratory 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 July 1, 2021, BioFire Defense **predicts 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** lists the current VOCs and VOIs as reported by the CDC and WHO.

**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 | UK |
| | B.1.351 | Beta | South Africa |
| | P.1 | Gamma | Brazil |
| | B.1.617.2 | Delta | India |
| **Variants of Interest** | B.1.525 | Eta | US (NY) |
| | B.1.526 | Iota | US (NY) |
| | B.1.617, B.1.617.1, B.1.617.3 | Kappa, Other | India |
| | P.2 | Zeta | Brazil |
| | B.1.427 | Epsilon | US (CA) |
| | B.1.429 | Epsilon | US (CA) |
| | C.37 | Lambda | Peru |

Variants of Concern and Variants of Interest are derived from the CDC and the WHO on July 1, 2021



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BioFire Defense, LLC
www.BioFireDefense.com

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BioFire Defense performs regular *in silico* analyses to monitor for SARS-CoV-2 viruses predicted to have reduced reactivity for the COVID-19 Test. The analysis considers currently recognized VOCs and VOIs as well as yet-to-be classified lineages gaining prominence globally. To perform the analysis, SARS-CoV-2 whole genome sequences deposited in the GISAID EpiCov™ database were evaluated from recently collected patient samples (a total of 497,598 GISAID sequences collected from April through June 2021). These sequences capture strains likely to be currently circulating and offer the best picture of how SARS-CoV-2 is evolving through human transmission.

The built-in assay redundancy of the COVID-19 Test reduces the risk of false negatives because viruses must present multiple co-occurring mutations to impact test performance. Additionally, sequences with mutations falling within 10 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., mutations co-occurring on multiple assays and their location on the 3' end of primer) 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 April to June 2021**

| Assays affected | Sequences containing mutations within 10 bp of the 3' end of the primer across multiple assays # of Sequences (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Variants of Concern (VOCs) | | | | VOIs | Other | All sequences |
| | Alpha | Beta | Gamma | Delta | | | |
| **2a & 2d** | 50 (0.01%) | 0 (0.00%) | 0 (0.00%) | 4 (0.01%) | 0 (0.00%) | 1 (0.00%) | 55 (0.01%) |
| **2d & 2e** | 194 (0.06%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 7 (0.03%) | 9 (0.03%) | 210 (0.04%) |
| **2a & 2e** | 27 (0.01%) | 0 (0.00%) | 0 (0.00%) | 1 (0.00%) | 2 (0.01%) | 19 (0.07%) | 49 (0.01%) |
| **All assays** | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) |
| **Total # sequences (% of analysis)** | **349,407 (70.2%)** | **5,729 (1.2%)** | **22,973 (4.6%)** | **71,556 (14.4%)** | **20,000 (4.0%)** | **27,993 (5.6%)** | **497,598 (100%)** |

Assay names correspond to SARS-COV-2a (2a), SARS-COV-2d (2d), and SARS-CoV-2e (2e)
Non-ambiguous mutations under the primer binding regions that fall within 10bp of 3' end of the primer are considered in this analysis.

The high-risk mutations summarized in **Table 2** must be found at a greater than 5% frequency in the database for reporting, per FDA guidance. Therefore, the very low frequencies of these sequences in our analysis suggest an overall very low risk of false negatives for the BioFire COVID-19 Test among currently circulating strains.

## Conclusion

Global *in silico* analysis (up until July 1, 2021) predicts **no impacts to test performance of the BioFire® COVID-19 Test including reliable detection of VOCs and VOIs defined by the CDC and WHO on July 1, 2021**.

## 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. If you have any questions or concerns about this process, 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

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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.