

BioFire® Respiratory Panels (RP2.1, RP2.1*plus* and RP2.1-EZ) SARS-CoV-2 Reactivity

Introduction

The BioFire RP2.1, RP2.1*plus* and RP2.1-EZ are multiplexed nucleic acid tests intended for use with BioFire® FilmArray® Systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. This includes the detection of SARS-CoV-2 which is identified with two independent assays: SARSCoV2-1 which targets the S (Spike) gene and SARSCoV2-2 which targets the M (Membrane) gene. A positive result from either assay will result in a SARS-CoV-2 Detected result.

Note: BioFire tests do not report cycle threshold (Ct) values and the BioFire RP2.1 SARS-CoV-2 assays are not intended to monitor for novel mutations.

Global in silico SARS-CoV-2 Variant Analysis

BioFire has performed periodic updates of the in silico analysis provided in the BioFire RP2.1, RP2.1*plus*, and RP2.1-EZ Instructions for Use (IFU) based on available sequences in the GISAID database with the most recent performed on October 21, 2021 as shown in Table 1 below. BioFire has also performed a one month in silico analysis of the most currently deposited GISAID sequences (September 22, 2021 to October 21, 2021) in Table 2 below.

Table 1. In silico Prediction of SARS-CoV-2 Detection by BioFire SARSCoV2-1 and SARSCoV2-2 Assays (December, 2019 to October 21, 2021)

+/+ indicates detected by both assays with no impairment, +/- indicates detection by one assay with no impairment and potential for impaired detection by the other assay, -/- indicates potential for impaired detection by both assays

Predicted Assay Result		SARSCoV2-1 (S-gene)		# (%) sequences predicted to be detected with no limitations (one or both assays positive)
		+	-	
SARSCoV2-2 (M-gene)	+	3,906,468	78,732	4,005,015/4,005,158 (99.99%)*
	-	19,815	143*	

*One hundred and forty-three (81 unique) sequences have mismatches in the 3' half of primer(s) for both the SARSCoV2-1 and SARSCoV2-2 assays or mismatches in the 3' half of the SARSCoV2-1 assay and a 9 base pair deletion in the SARSCoV2-2 assay. The mismatches are predicted to impair detection at low analyte concentration.

Table 2. Single Month In silico Prediction of SARS-CoV-2 Detection by BioFire SARSCoV2-1 and SARSCoV2-2 Assays (September 22, 2021 to October 21, 2021)

+/+ indicates detected by both assays with no impairment, +/- indicates detection by one assay with no impairment and potential for impaired detection by the other assay, -/- indicates potential for impaired detection by both assays

Predicted Assay Result		SARSCoV2-1 (S-gene)		# (%) sequences predicted to be detected with no limitations (one or both assays positive)
		+	-	
SARSCoV2-2 (M-gene)	+	693,310	18,132	717,375/717,420 (99.99%)*
	-	5,933	45*	

*Forty-five (32 unique under the primers) sequences have mismatches in the 3' half of primer(s) for both the SARSCoV2-1 and SARSCoV2-2 assays. The mismatches are predicted to impair detection at low analyte concentration.

The number of sequences analyzed and the number of sequences that are predicted to impair detection at low analyte concentrations in the September 22, 2021 to October 21, 2021 single month analysis are comparable to the June 22, 2021 to July 21, 2021 numbers, suggesting stable performance.

This analysis indicates that the BioFire RP2.1 family of products will be able to amplify and detect 100% of sequences retrieved on October 21, 2021. The analysis includes sequences from the lineages listed below

- A.23 lineage (Uganda)
- A.27 (France) / HMN.19B
- B.1 + 214insQAS
- B.1.1.7 / VOC-20DEC-01 / Alpha (United Kingdom)
 - B.1.1.7 + S494P (United Kingdom)
 - B.1.1.7 + Q677H (United Kingdom)
 - Q.1
 - Q.2
 - Q.3
 - Q.4
 - Q.5
 - Q.6
 - Q.7
 - Q.8
- B.1.1.28 and descendants
 - P.1 lineage / VOC-21JAN-02 variant / Gamma (Brazil)
 - P.1.1
 - P.1.2
 - P.1.4
 - P.1.6
 - P.1.7
 - P.2 lineage / VUI-21JAN-01 variant / Zeta (Brazil)
 - P.3 lineage / VUI-21MAR-02 / Theta (Philippines/Japan)
 - B.1.1.28 + N501T + E484Q (Brazil)
- B.1.1.318 lineage / VUI-21FEB-04 variant (United Kingdom)
- B.1.214 lineage / Belgium variant (Belgium)
- B.1.214.2 (Belgium)
- B.1.243.1 / Arizona variant (United States)
- B.1.351 lineage / VOC-20DEC-02 variant / Beta (South Africa)
 - B.1.351.2
 - B.1.351.3
- B.1.427/B.1.429 lineage / CAL.20C variant / Epsilon (United States)
- B.1.525 / VUI-21FEB-03 variant / Eta (United Kingdom)
- B.1.526 / Iota (United States)
- B.1.526.1 (United States)
- B.1.616 / Breton variant (France)
- B.1.617.1 / VUI-21APR-02 / Kappa (India)

- B.1.617.2 / VOC-21APR-02 / Delta (India)
 - AY.1
 - AY.2
 - AY.3
 - AY.3.1
 - AY.4
 - AY.4.1
 - AY.4.2
 - AY.4.3
 - AY.4.4
 - AY.4.5
 - AY.5
 - AY.5.1
 - AY.5.2
 - AY.6
 - AY.7
 - AY.7.1
 - AY.7.2
 - AY.8
 - AY.9
 - AY.10
 - AY.11
 - AY.12
 - AY.13
 - AY.14
 - AY.15
 - AY.16
 - AY.17
 - AY.18
 - AY.19
 - AY.20
 - AY.21
 - AY.22
- AY.23
 - AY.23.1
- AY.24
- AY.25
- AY.26
- AY.27
- AY.28
- AY.29
 - AY.29.1
- AY.30
- AY.31
- AY.32
- AY.33
- AY.34
- AY.35
- AY.36
- AY.37
- AY.38
- AY.39
 - AY.39.1
 - AY.39.1.1
- AY.40
- AY.41
- B.1.617.3 / VUI-21APR-03 (India)
- B.1.620
- B.1.621
 - B.1.621.1
- C.36
- C.36.3 / VUI-21MAY-02 (Thailand ex Egypt)
- C.37 / B.1.1.1 + L452Q + F490S / Lambda
- R.1
- AV.1 / VUI-21MAY-01

Additional in-silico analysis was performed on November 29, 2021 regarding Variant of Concern (VOC) B.1.1.529/Omicron. Analysis indicates that the BioFire RP2.1 family of products will be able to amplify and detect 100% of the currently available B.1.1.529/Omicron sequences.

The one hundred and forty-three very rare sequences represent only eighty-one unique sequences that indicate a potential for impaired detection by both assays (indicated in Table 1). Fifty-six of eighty-one unique sequences have been evaluated using synthetic nucleic acid template to estimate the impact of the observed mismatches on amplification and detection by both assays. Table 3 below shows the observed effect of the mismatches found in the 56 unique sequences tested with synthetic templates. The BioFire RP2.1, RP2.1*plus* and RP2.1-EZ SARS-CoV-2 test only requires one assay to be positive in order to report “SARS-CoV-2 Detected” therefore these one hundred and forty-three very rare sequences are expected to be detected by the BioFire RP2.1 family of products but could demonstrate a reduction in analytical sensitivity near the limit of detection.

Table 3. Results of completed synthetic template testing of sequences with possible impairment in both SARS-CoV-2 assays.

Effect on pouch	Number of unique sequences tested	Total number of sequences
No effect	24 / 56	49 / 4,005,158
Minor (2-10 fold reduction)	19 / 56	45 / 4,005,158
Mild (10-100 fold reduction)	10 / 56	15 / 4,005,158
Moderate (≥ 100 fold reduction)	3 / 56	4 / 4,005,158

This analysis supports the conclusion that all of the 4,005,158 sequences evaluated as of October 21, 2021 can be amplified and detected by the BioFire RP2.1 family of tests, though a limitation or impairment on detection is predicted at low concentrations ($\leq 10x$ the limit of detection) for 0.0036% of the sequences (143/4,005,158) with only thirteen unique sequences identified with detection likely affected greater than 10 fold.

Conclusions

1. The BioFire Respiratory 2.1 Panels (RP2.1, RP2.1*plus* and RP2.1-EZ) SARS-CoV-2 assays are not affected by any circulating SARS-CoV-2 lineages identified as of October 21, 2021 as well as B.1.1.529/Omicron (as of November 29, 2021).
2. Global in silico analysis (as of October 21, 2021) predicts that the BioFire Respiratory Panels (RP2.1, RP2.1*plus* and RP2.1-EZ) SARS-CoV-2 assays will detect all sequences evaluated.
3. BioFire tests do not report cycle threshold (Ct) values and the BioFire RP2.1 SARS-CoV-2 assays are not intended to monitor for novel mutations.

Bioinformatics for the SARS-CoV-2 virus is expanding at a rapid rate since the emergence of the virus in human infection in late 2019. Thousands of viral whole genome sequences are being evaluated and submitted to public and private databases on a monthly basis. As the pandemic persists and viral genomes evolve, monitoring of assay reactivity with new sequences is important for understanding the state-of-the-art for performance of the SARS-CoV-2 assays in the BioFire RP2.1 family of products (RP2.1, RP2.1*plus* and RP2.1-EZ).

BioFire continues to monitor these new sequences and is performing regular in silico analyses of the RP2.1 family SARS-CoV-2 assays.

Technical Support Contact Information

BioFire 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.

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