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 July 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 (June 22, 2021 to July 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 July 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 / # sequences		SARSCoV2-1 (S-gene) +	SARSCoV2-1 (S-gene) -	# (%) sequences predicted to be detected with no limitations (one or both assays positive)
SARSCoV2-2 (M-gene)	+	2,078,776	35,566	2,118,581/2,118,638 (99.99%)*
	-	4,239	57*	

*Fifty-seven (35 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.

Technical Note
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Table 2. Single Month In silico Prediction of SARS-CoV-2 Detection by BioFire SARSCoV2-1 and SARSCoV2-2 Assays (June 22, 2021 to July 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 / # sequences		SARSCoV2-1 (S-gene)		# (%) sequences predicted to be detected with no limitations (one or both assays positive)
		+	-	
SARSCoV2-2 (M-gene)	+	344,617	7,911	353,534/353,546 (99.99%)*
	-	1,006	12*	

*Twelve (9 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 June 22, 2021 to July 21, 2021 single month analysis are comparable to the April 22, 2021 to May 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 July 21, 2021. The analysis includes sequences from the lineages listed below

- **B.1.351 lineage / VOC-20DEC-02 variant / Beta (South Africa)**
  - **B.1.351.2**
  - **B.1.351.3**
- **B.1.1.28.1 (or P.1) lineage / VOC-21JAN-02 variant / Gamma (Brazil)**
  - **P.1.1**
  - **P.1.2**
- **B.1.1.28.2 (or P.2) lineage / VUI-21JAN-01 variant / Zeta (Brazil)**
- **B.1.1, B.1.1.7, & B.1.258 lineages with Δ69-70 and N501Y (United Kingdom)**
- **B.1.429/B.1.427 lineage / CAL.20C variant / Epsilon (United States)**
- **B.1.1.7 lineage / VOC-20DEC-01 variant / Alpha (United Kingdom)**
- **B.1.1.7 + E484K / VOC-21FEB-02 variant (United Kingdom)**
- **B.1.525 / VUI-21FEB-03 variant / Eta (United Kingdom)**
- **B.1.526 / Iota (United States)**
- **B.1.1.318 lineage / VUI-21FEB-04 variant (United Kingdom)**
- **B.1.1.28.3 (or P.3) lineage / VUI-21MAR-02 variant / Theta (Philippines)**
- **B.1.214 lineage / Belgium variant (Belgium)**
- **B.1.616 / Breton variant (France)**
- **HMN 19.B (France)**
- **A.23.1 + E484K / VUI-21FEB-01 (United Kingdom)**
- **B.1.617.1 + E484Q / VUI-21APR-01 /Kappa (India)**
- **B.1.617.2 / VOC-21APR-02 / Delta (India)**
  - **AY.1**
  - **AY.2**
- **B.1.617.3 / VUI-21APR-03 (India)**
- **B.1.1.7 + S494P (United Kingdom)**
- **A.27 (France)**

- **B.1.1.7 + Q677H (United Kingdom)**
- **B.1.620 (TBC)**
- **B.1.214.2 (Belgium)**
- **B.1.1.28 + N501T + E484Q (Brazil)**
- **C.36 (TBC)**
- **B.1.621 (TBC)**
- **B.1 + 214insQAS (TBC)**
- **B.1.243.1 / Arizona variant (United States)**
- **B.1.526.1 (United States)**
- **AV.1 / VUI-21MAY-01 (TBC)**
- **R.1 (TBC)**
- **C.37 / Lambda (Peru)**
- **C.36.3 / VUI-21MAY-02 (Thailand ex Egypt)**

The fifty-seven very rare sequences represent only thirty-five unique sequences that indicate a potential for impaired detection by both assays (indicated in Table 1). Twenty-eight of thirty-five 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 28 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 fifty-seven very rare sequences are expected to be detected by the BioFire RP2.1 family of products but could demonstrate a mild 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	10 / 28	15 / 2,118,638
Minor (2-10 fold reduction)	13 / 28	23 / 2,118,638
Mild (≥10 fold reduction)	5 / 28	9 / 2,118,638

This analysis supports the conclusion that all of the 2,118,638 sequences evaluated as of July 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 (≤10x the limit of detection) for 0.0027% of the sequences (57/2,118,638) with only five 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 July 21, 2021.
2. Global in silico analysis (as of July 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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