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 March 21, 2021 as shown in Table 1 below. BioFire has also performed a one month snapshot in silico analysis of the most currently deposited GISAID sequences (Feb 22, 2021 to March 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 March 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)	+	658,401	7,636	667,251/667,270 (99.99%)*
	-	1,214	19*	

*Nineteen (14 unique) 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.

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Table 2. Single Month In silico Prediction of SARS-CoV-2 Detection by BioFire SARSCoV2-1 and SARSCoV2-2 Assays (Feb 22, 2021 to March 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)	+	224,663	3,232	228,407/228,419 (99.99%)*
	-	512	12*	

*Twelve (8 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.

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

- **B.1.351 lineage / VOC-20DEC-02 variant (South Africa)**
- **B.1.1.28.1 (or P.1) lineage / VOC-21JAN-02 variant (Brazil)**
- **B.1.1.28.2 (or P.2) lineage / VUI-21JAN-01 variant (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 (United States)**
- **B.1.1.7 lineage / VOC-20DEC-01 variant (United Kingdom)**
- **B.1.1.7 + E484K / VOC-21FEB-02 variant (United Kingdom)**
- **B.1.525 / VUI-21FEB-03 variant (United Kingdom)**
- **B.1.526 (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 (Philippines)**
- **B.1.214 lineage / Belgium variant (Belgium)**
- **B.1.616 / Breton variant (France)**
- **HMN 19.B (France)**

The nineteen very rare sequences represent only fourteen unique sequences that indicate a potential for impaired detection by both assays (indicated in Table 1). One of these fourteen unique sequences is a member of the B.1.1.7 lineage listed above. So far 5/14 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. Due to the redundant nature of the SARSCoV2-1 and SARSCoV2-2 assays, four of the five tested unique sequences show little (2-4 fold) to no impact on amplification and detection relative to the control sequence. One unique sequence (1/667,270) shows a minor (5-10 fold) impact to amplification and detection relative to the control sequence. The unique sequence that is a member of lineage B.1.1.7 has yet to be tested. 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 nineteen 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.

This analysis supports the conclusion that all 667,270 sequences evaluated as of March 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 0.0028% of the sequences (19/667,270).

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 March 21, 2021.
2. Global in silico analysis (as of March 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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