BIOFIRE® Respiratory Panels (RP2.1, RP2.1plus 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 June 21, 2022 as shown in Table 1 below. BIOFIRE has also performed a one month in silico analysis of the most currently deposited GISAID sequences (May 22, 2022 to June 21, 2022) 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 June 21, 2022)

+/+ 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)	+	9,098,099	156,400	9,292,138/9,292,487 (99.996%)*
	-	37,639	349*	

*Three hundred and forty-nine (134 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.

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Table 2. Single Month In silico Prediction of SARS-CoV-2 Detection by BIOFIRE SARSCoV2-1 and SARSCoV2-2 Assays (May 22, 2022 to June 21, 2022)
+/+ 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)	+	409,842	3,550	414,549/414,552 (99.999%)*
	-	1,157	3*	

*Three (2 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 May 22, 2022 to June 21, 2022 single month analysis are comparable to the January 22, 2021 to February 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 June 21, 2022. 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.1.1.529 lineage / Omicron
- B.1.214 lineage / Belgium variant (Belgium)
- B.1.214.2 (Belgium)
- B.1.243.1 / Arizona variant (United States)

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- 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.3.2
    - AY.3.3
    - AY.3.4
  - AY.4
    - AY.4.1
    - AY.4.2
      - AY.4.2.1
      - AY.4.2.2
      - AY.4.2.3
      - AY.4.2.4
      - AY.4.2.5
    - AY.4.3
    - AY.4.4
    - AY.4.5
    - AY.4.6
    - AY.4.7
    - AY.4.8
    - AY.4.9
    - AY.4.10
    - AY.4.11
    - AY.4.12
    - AY.4.13
    - AY.4.14
    - AY.4.15
    - AY.4.16
    - AY.4.17
  - AY.5
    - AY.5.1
    - AY.5.2
    - AY.5.3
    - AY.5.4
    - AY.5.5
    - AY.5.6
    - AY.5.7
  - AY.6
  - AY.7
    - AY.7.1
    - AY.7.2
  - AY.8
  - AY.9
    - AY.9.1
    - AY.9.2
      - AY.9.2.1
      - AY.9.2.2
  - AY.10
  - AY.11
  - AY.12
  - AY.13
  - AY.14
  - AY.15
  - AY.16
    - AY.16.1
  - AY.17
  - AY.18
  - AY.19
  - AY.20
    - AY.20.1
  - AY.21
  - AY.22
  - AY.23
    - AY.23.1
    - AY.23.2
  - AY.24
    - AY.24.1
  - AY.25
    - AY.25.1
      - AY.25.1.1
      - AY.25.1.2
    - AY.25.2
    - AY.25.3

- AY.26
  - AY.26.1
- AY.27
- AY.28
- AY.29
  - AY.29.1
  - AY.29.2
- AY.30
- AY.31
- AY.32
- AY.33
  - AY.33.1
  - AY.33.2
- AY.34
  - AY.34.1
    - AY.34.1.1
  - AY.34.2
- AY.35
- AY.36
  - AY.36.1
- AY.37
- AY.38
- AY.39
  - AY.39.1
    - AY.39.1.1
    - AY.39.1.2
    - AY.39.1.3
    - AY.39.1.4
  - AY.39.2
  - AY.39.3
  - AY.39.4
- AY.40
- AY.41
- AY.42
  - AY.42.1
- AY.43
  - AY.43.1
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- AY.45
- AY.46
  - AY.46.1
  - AY.46.2
  - AY.46.3
  - AY.46.4
  - AY.46.5
  - AY.46.6
    - AY.46.6.1
- AY.47
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- AY.75
  - AY.75.2
  - AY.75.3
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- AY.79
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- AY.83
- AY.84
- AY.85
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- AY.87
- AY.88
- AY.89
- AY.90
- AY.91
  - AY.91.1
- AY.92
- AY.93
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- AY.99
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- AY.101
- AY.102
- AY.103
  - AY.103.1
  - AY.103.2
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- AY.106
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- AY.109
- AY.110
- AY.111
- AY.112
  - AY.112.1
  - AY.112.2
  - AY.112.3
- AY.113
- AY.114
- AY.115
- AY.116
  - AY.116.1
- AY.117
- AY.118
- AY.119
  - AY.119.1
  - AY.119.2
- AY.120
  - AY.120.1
  - AY.120.2
    - AY.120.2.1
- AY.121
  - AY.121.1
- AY.122
  - AY.122.1
  - AY.122.2
  - AY.122.3
  - AY.122.4
  - AY.122.5
  - AY.122.6
- AY.123
  - AY.123.1
- AY.124
  - AY.124.1
    - AY.124.1.1
- AY.125
  - AY.125.1
- AY.126
- AY.127
  - AY.127.1
  - AY.127.2
  - AY.127.3
- AY.128
- AY.129
- AY.130
- AY.131
- AY.132
- AY.133
- AY.134

- B.1.1.529 / BA.1 / Omicron
  - BA.1.1
    - BA.1.1.1
    - BA.1.1.2
    - BA.1.1.3
    - BA.1.1.4
    - BA.1.1.5

        - BA.1.1.6
        - BA.1.1.7
        - BA.1.1.8
        - BA.1.1.9
        - BA.1.1.10
        - BA.1.1.11
        - BA.1.1.12
        - BA.1.1.13
        - BA.1.1.14
        - BA.1.1.15
        - BA.1.1.16
        - BA.1.1.17
        - BA.1.1.18
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    - BA.1.12
    - BA.1.13
        - BA.1.13.1
    - BA.1.14
        - BA.1.14.1
        - BA.1.14.2
    - BA.1.15
        - BA.1.15.1
        - BA.1.14.2
    - BA.1.16
        - BA.1.16.1
        - BA.1.16.2
    - BA.1.17
        - BA.1.17.1
        - BA.1.17.2
    - BA.1.18
    - BA.1.19
    - BA.1.20
    - BA.1.21
        - BA.1.21.1
    - BA.1.22
- BA.2
    - BA.2.1
    - BA.2.2
    - BA.2.3
        - BA.2.3.1
        - BA.2.3.2
        - BA.2.3.3
        - BA.2.3.4
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        - BA.2.9.1
        - BA.2.9.2
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        - BA.2.10.1
    - BA.2.11
    - BA.2.12
        - BA.2.12.1
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    - BA.2.19
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    - BA.2.21
    - BA.2.22
    - BA.2.23
    - BA.2.24
    - BA.2.25
        - BA.2.25.1
    - BA.2.26
    - BA.2.27
    - BA.2.28
    - BA.2.29
    - BA.2.30
    - BA.2.31
    - BA.2.32
    - BA.2.33
    - BA.2.34

  - BA.3
    - BA.3.1
  - BA.4
  - BA.5
- 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
- XD
- XE
- XF

The three hundred and forty-nine very rare sequences represent only one hundred and thirty-four unique sequences that indicate a potential for impaired detection by both assays (indicated in Table 1). Eighty-two of one hundred and thirty-four 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 82 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 three hundred and forty-nine 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	36 / 82	119 / 9,292,487
Minor (2-10 fold reduction)	35 /82	135 / 9,292,487
Mild (10-100 fold reduction)	10 / 82	16 / 9,292,487
Moderate (≥100 fold reduction)	1 / 82	1 / 9,292,487

This analysis supports the conclusion that all of the 9,292,487 sequences evaluated as of June 21, 2022 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 less than 0.004% of the sequences (349/9,292,487) with only eleven 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 June 21, 2022.
2. Global in silico analysis (as of June 21, 2022) 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.

Note: The BIOFIRE RP2.1-EZ test is for in vitro diagnostic use under Emergency Use Authorization only.

BIOFIRE RP2.1-EZ has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high, moderate, or waived complexity tests. This product is for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

BIOFIRE RP2.1-EZ has been authorized only for the detection and differentiation of nucleic acid of SARS-CoV-2 from multiple respiratory viral and bacterial organisms.

The emergency use of BIOFIRE RP2.1-EZ is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics 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.

Technical Support Contact Information

bioMérieux 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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Phone: +1-801-736-6354, select Option 5