POSTER 1403

Clinical Evaluation of the BioFire® COVID-19 Test 2

Agnieszka Lewandowska, Mark A. Gurling, Jared R. Helm, Wendy Smith, Diane Walker, Pascal Belgique, Lia Gale, Olivia Jackson, Michael Johnson, Alex Kelley, Ashlie Stephenson, Hannah VanHollebeke, Sidney Webster, Ashley Wiltsie, Marianne Kim, Cynthia Phillips, David S. Rabiger

BioFire Defense, LLC, Salt Lake City, Utah, USA

CONTACT INFORMATION

Agnieszka Lewandowska, PhD.
Agnieszka.Lewandowska@BioFireDefense.com

David Rabiger, PhD.
David.Rabiger@BioFireDefense.com

ABSTRACT

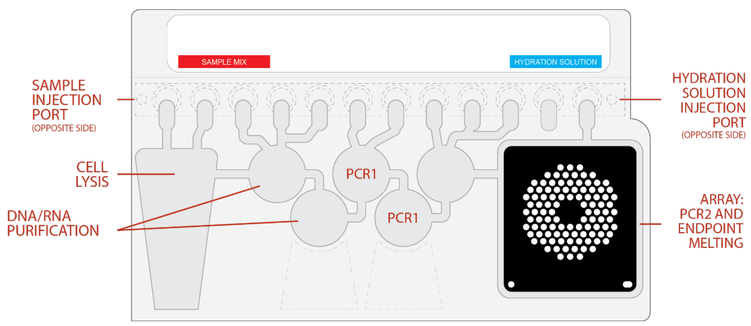
Coronavirus Disease 2019 (COVID-19) is a contagious respiratory illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Diagnosis of COVID-19 is critical to public health efforts to identify infectious individuals and reduce the spread of disease. The BioFire COVID-19 Test 2 was developed by BioFire Defense LLC in cooperation with the US Department of Defense (Contract Nos. W81XWH20C0076 and W81XWH21C0003) and has been cleared by the US FDA. The test targets select regions of the SARS-CoV-2 genome and is run on the easy-to-use BioFire® FilmArray® platform. Following the initial 510(k) clearance of the BioFire COVID-19 Test 2 targeting three regions of SARS-CoV-2 genome, a subsequent development effort allowed to expand to a total of seven assays interpreted by the panel software in the version cleared by the FDA in July 2022 (K221460). Analytical sensitivity of the BioFire COVID-19 Test 2 was determined to be 3.3E+02 GE/mL with inactivated SARS-CoV-2 virus demonstrating that the BioFire COVID-19 Test 2 is a highly sensitive molecular test for the detection of SARS-CoV-2. A prospective clinical study at three different sites within the US evaluated performance of the BioFire COVID-19 Test 2 using nasopharyngeal swab specimens collected from individuals suspected of COVID-19 and the BioFire Respiratory Panel 2.1 as a comparator. The positive percent agreement (PPA) was determined to be 98.6% PPA and the negative percent agreement (NPA) was determined to be 99.1% for the version utilizing all seven assays.Due to the continued evolution of the SARS-CoV-2 genome, BioFire Defense monitors currently circulating variants. An *in silico* analysis predicted that >99.9% of variant sequences (as of September 13, 2022) have perfect complementarity to three or more of the seven assays. These data indicate that deploying additional four assays maintains sensitivity and specificity while ensuring robust detection of evolving SARS-CoV-2 virus variants with the BioFire COVID-19 Test 2.

INTRODUCTION

Global spread of Coronavirus Disease 2019 (COVID-19) created an abrupt need to quickly identify infections. On 2020-02-04 the Secretary of the Department of Health and Human Services (HHS) declared that COVID-19 constituted a public health emergency with a significant potential to affect national security or the health and security of United States citizens living abroad, and authorized emergency use of in vitro diagnostics for its detection and/or diagnosis. BioFire Defense (BFDf) received an initial emergency use authorization (EUA) for its newly developed COVID-19 test on 2020-03-23. Subsequent efforts, including additional analytical and clinical testing, led to the expansion of the EUA version of the test to multiple sample types, validation of additional assays within the test and a push to obtain an in vitro diagnostic device FDA clearance.

The test is an easy-to-use, "lab-in-a-pouch" device utilizing an automated, multiplex PCR BioFire FilmArray system to perform nucleic acid purification, reverse transcription, nested multiplex PCR amplification and DNA melting (Figure 1) to analyze samples for the presence of SARS-CoV-2 RNA. The FilmArray system (BioFire FilmArray 2.0 or BioFire FilmArray Torch) relies on a closed system disposable pouch that stores all the reagents necessary for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection (Figure 1). After sample collection, the user injects hydration solution on one side of the pouch and sample combined with sample buffer into the other side of the pouch, places the pouch into a FilmArray instrument, and starts a run. Loading the pouch takes about 2 minutes, and the entire run process takes about 45 minutes (for the BioFire COVID-19 Test 2).

FIGURE 1. BIOFIRE FILMARRAY - AN AUTOMATED MULTIPLEX PCR SYSTEM



During a Run, the FilmArray System:

- Lyses the sample by agitation (bead beating).
- Extracts and purifies all nucleic acids from the sample using magnetic bead technology.
- Performs nested multiplex PCR by: a single, large volume, highly multiplexed first-stage PCR reaction (PCR1), followed by a multiple, nested singleplex second-stage PCR reactions (PCR2) to amplify sequences within the PCR1 products.
- Uses endpoint melting curve data to detect and generate a result for each target on the array.

SPECIMEN COLLECTION AND STUDY SITES

Clinical study sites (**Table 1**) were chosen based on their expertise in performing SARS-CoV-2 diagnostics and ability to conduct the study in accordance with the protocol and Good Clinical Practices (GCP). The specimens were nasopharyngeal swabs (NPS) in transport medium, collected from subjects of all ages, that were leftovers following standard of care (SoC) testing for SARS-CoV-2 (by an assay that included an extraction step and that had received an EUA designation). The use of deidentified leftover clinical specimens was exempt from the requirement to obtain informed consent, and approved by IRBs. Study sites represented different regions of the US, and specimens were collected over 4 months in 2020 (July – October). The study included a diverse patient population, with approximately equal numbers of females enrolled compared to males (48.4% and 51.1%, respectively). Study enrollees were predominantly adults (only 10.5% were 0-18 years).

TABLE 1. STUDY SITES AND SUBJECT DEMOGRAPHICS

		Site 1	Site 2	Site 3	Overall
Site Details					
Location		Tampa General Hospital	Northwell Health Laboratories	Loyola University Medical Center	
Enrollment Dates		07/2020 – 10/2020	09/2020 – 10/2020	09/2020 – 10/2020	07/2020 – 10/2020
Specimens Selected (excluded)		312 (0)	110 (0)	112 (7)	534 (7)
Valid Test Results (invalid)		310 (2)	108 (2)	105 (0)	523 (4)
Demographics					
Sex	Female	141 (45.5%)	54 (50.0%)	58 (55.2%)	253 (48.4%)
	Male	169 (54.5%)	51 (47.2%)	47 (44.8%)	267 (51.1%)
	Unknown	0 (0%)	3 (2.8%)	0 (0%)	3 (0.6%)
Age Range	0-18 years	24 (7.7%)	18 (16.7%)	13 (12.4%)	55 (10.5%)
	19-40 years	102 (32.9%)	45 (41.7%)	23 (21.9%)	170 (32.5%)
	41-60 years	94 (30.3%)	32 (29.6%)	20 (19.0%)	146 (27.9%)
	61+ years	90 (29.0%)	13 (12.0%)	49 (46.7%)	152 (29.1%)

BIOFIRE COVID-19 TEST 2 CLINICAL PERFORMANCE

In the clinical evaluation, the BioFire COVID-19 Test 2 was highly specific (Negative Percent Agreement, NPA of 99.1%) and sensitive (Positive Percent Agreement, PPA of 98.6%) when compared to the predicate device, BioFire Respiratory Panel 2.1 (RP2.1) (**Table 2**). RP2.1 panel is intended for detection of multiple respiratory pathogens, and includes 2 assays for SARS-CoV-2. A follow up analysis of the discrepant results showed that three (3/4) of the false positive specimens showed evidence of SARS-CoV-2 by Central Reference Laboratory EUA testing and all four appeared to have been a consequence of analyte levels near the limit of detection (LoD) of the BioFire RP2.1 (comparator) assays. The single false negative specimen did not show evidence of SARS-CoV-2 by standard of care testing or follow up comparator EUA testing, and was likely due to analyte level being near the LoD of BioFire COVID-19 Test 2 and other assays.

TABLE 2. BIOFIRE COVID-19 TEST 2 PERFORMANCE EVALUATED IN REFERENCE TO THE COMPARATOR DEVICE

		BioFire RP2.1		BioFire COVID-19 Test 2 Performance		
		Positive	Negative		Performance	95% Confidence Interval
BioFire COVID-19 Test 2	Positive	68	4	Sensitivity (positive percent agreement, PPA)	68/69 (98.6%)	92.2-99.7%
	Negative	1	450	Selectivity (negative percent agreement, NPA)	450/454 (99.1%)	97.8-99.7%
	Total	69	454			

The FilmArray instrument and BioFire COVID-19 Test 2 pouches have a low failure rate. The success rate for the FilmArray instrument in initial specimen tests during the clinical evaluation was 99.6% (not shown). The success rate for pouch controls during the clinical evaluation was 99.8% (**Table 3**).

TABLE 3. ANALYSIS OF POUCH CONTROL PERFORMANCE

Initiated Runs						
Subtotal	Subtotal	Pouch Controls Passed	Completed Runs: Pouch Controls Failed			
		Subtotal	Subtotal	PCR2 Control and RNA Process Control Failed	Only PCR2 Control Failed	Only RNA Process Control Failed
526	524	523 / 99.8%	1 / 0.2%	0 / 0.0%	0 / 0.0%	1 / 0.2%

LIMIT OF DETECTION AND STRAIN INCLUSIVITY

BioFire COVID-19 Test 2 sensitivity was analyzed using both infectious material and inactivated virus. The limit of detection (LoD) was determined to be 3.3E+02 genomic copies/ml, and comparable for both sample types (**Table 4**). BioFire COVID-19 Test 2 was also evaluated against the FDA SARS-CoV-2 Reference Panel using spiked NPS specimens (LoD of 5,400 NDU/mL) and tested with additional four virus variants available at the time (Chile/Santiago_op4d1/2020 (BEI/NR-52439), Hong Kong/VM20001061/2020 (BEI/NR-52282), Italy-INMI1 (BEI/NR-52284) and New York-PV08410/2020 (BEI/NR-53514)). There were no sensitivity limitations for the variants tested.

TABLE 4. SARS-COV-2 LOD TEST RESULTS FOR THE BIOFIRE COVID-19 TEST

Variant (Source)	LoD Concentration		# Detected/Total (% Detection)
	Genomic Copies/mL	TCID50/mL	
USA-WA1/2020 (infectious, WRCEVA)	3.3E+02	2.2E-02	20/20 (100%)
USA-WA1/2020 (heat-inactivated; BEI NR-52286)	3.3E+02	4.3E-02	20/20 (100%)

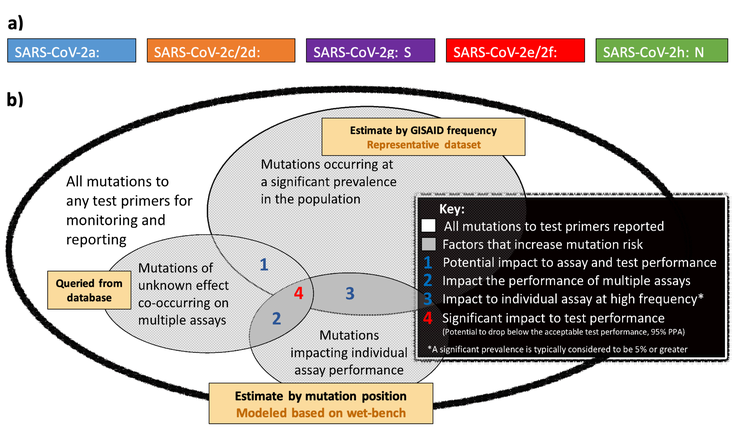
INCLUSIVITY MONITORING *in silico*

The BioFire COVID-19 Test 2 consists of seven assays targeting different regions of the viral genome (**Figure 2a**). The use of seven assays reduces the risk of false negatives from emerging variants as co-occurring mutations affecting the primer binding regions of multiple assays are rare. BioFire Defense performs regular *in silico* monitoring of available variant sequences to identify SARS-CoV-2 viruses for which the BioFire COVID-19 Test may have reduced reactivity.

A combination of several factors is needed to create a high-risk scenario for performance (Figure 2b):

- A mutation that has significant risk of impacting an individual assay.
- A combination of mutations (of unknown, and possibly minor, effect) potentially affecting multiple assays at a time.
- A mutation occurring at a significant prevalence in the population.

FIGURE 2. MULTIPLE ASSAYS TARGETING DIFFERENT REGIONS OF SARS-COV-2 GENOME DECREASE THE RISK OF SINGLE MUTATIONS AFFECTING THE TEST'S PERFORMANCE.



The *in silico* analysis of the test's inclusivity performed by BioFire Defense considers currently recognized Variants of Concern (VOCs), Variants of Interest (VOI) and Variants and Subvariants Under Monitoring (VUMs and SUMs) as well as yet-to-be classified lineages gaining prominence globally.

Table 5 summarizes variants included in the most recent analysis. Full-length, human-host SARS-CoV-2 sequences with high coverage obtained from recently collected patient samples and deposited in the GISAID EpiCoV™ database are evaluated. These sequences capture strains likely to be circulating and offer the best picture of how SARS-CoV-2 is evolving through human transmission.

TABLE 5. SUBVARIANTS UNDER MONITORING (SUM) AND VARIANTS OF CONCERN (VOC)

Pangolin Lineage	WHO Variant Designation	June - August 2022 # (%)	August 2022 # (%)
Subvariants Under Monitoring (SUM)			
BA.2.12.1	Omicron	15,957 (25.06)	269 (3.34)
BA.2.75	Omicron	1,268 (1.99)	591 (7.34)
BA.4	Omicron	1,345 (2.11)	227 (2.82)
BA.5	Omicron	15,949 (25.04)	5,385 (66.9)
Variants of Concern (VOC)			
Recombinant[1]	Omicron	9 (0.01)	0
Non SUM	Omicron	25,764 (40.45)	465 (5.78)
Lineages Not Currently Under Monitoring by the WHO			
		3,394 (5.33)	1,112 (13.82)
All Sequences			
		63,686 (100)	**8,049 (100)**

[1] *All Recombinant lineages of Omicron sublineages (e.g., XE is a recombinant of BA.1 and BA.2)*

Note: Yellow: ≥5% change between periods

Note: Variants designated by the WHO on September 13th, 2022. There are currently no VOIs or VUMs designated.

Analysis results fora total of 63,686 GISAID sequences submitted before September 13, 2022, with collection dates from June 1, 2022 through August 31, 2022 are shown in **Table 6**.

TABLE 6. SUMMARY OF HIGHER RISK CO-OCCURRING MUTATIONS IN SEQUENCES COLLECTED FROM JUNE 1, 2022 TO AUGUST 31, 2022

Frequencies of Sequences with High-Risk Mutations[1]

Pangolin Lineage	Mutated Sequences by Assay: 2a	2c	2d	2e	2f	2g	2h	Co-occurring Mutated Sequences[2]: 5	≥6	Sequences by Lineage
Subvariants Under Monitoring (SUM)										
BA.2.12.1	202 (1%)	127 (1%)	115 (1%)	136 (1%)	169 (1%)	15,946 (100%)	99 (1%)	0	0	15,957 (25.1%)
BA.2.75	11 (1%)	4 (0%)	4 (0%)	12 (1%)	24 (2%)	1,266 (100%)	3 (0%)	1 (0%)	0	1,268 (2%)
BA.4	4 (0%)	4 (0%)	4 (0%)	17 (1%)	22 (2%)	1,343 (100%)	2 (0%)	1 (0%)	0	1,345 (2.1%)
BA.5	79 (0%)	137 (1%)	142 (1%)	193 (1%)	342 (2%)	15,892 (100%)	64 (0%)	1 (0%)	0	15,949 (25%)
Variants of Concern (VOC)										
Recombinant[3]	0	0	0	0	0	9 (100%)	0	0	0	9 (0%)
Non SUM	138 (1%)	283 (1%)	292 (1%)	235 (1%)	620 (2%)	25,699 (100%)	283 (1%)	1 (0%)	0	25,764 (40.5%)
Lineages Not Currently Under Monitoring by the WHO										
	7 (0%)	22 (1%)	27 (1%)	27 (1%)	38 (1%)	3,351 (99%)	10 (0%)	0	0	3,394 (5.3%)
Summary: All Sequences by Assays										
	441 (0.7%)	**577 (0.9%)**	**584 (0.9%)**	**620 (1%)**	**1,215 (1.9%)**	**63,506 (99.7%)**	**461 (0.7%)**	**4 (0%)**	**0**	**63,686 (100%)**

[1] *Non-ambiguous mutations under the primer binding regions that fall within 10bp of 3' end of the primer were considered in this analysis.*
[2] *Mutated sequences co-occurring on multiple assays, by # assays*
[3] *All Recombinant lineages of Omicron sublineages (e.g., XE is a recombinant of BA.1 and BA.2)*
Color guide - Yellow: mutations occur at ≥5%; Blue: mutations occur at < 1%
Note: Assay names are abbreviated as SARS-COV-2a (2a), SARS-COV-2d (2d), etc.

Inclusivity analysis predicts that currently circulating variants pose no risks to reactivity of the BioFire COVID-19 Test 2.

- No variants found with indels or >1 mismatch to test primer sequences at a prevalence of ≥0.1% in the sequence dataset
- Negligible (0%) number of variants with co-occurring mutations within 5 or more assays
- Only one variant identified (single mismatch for one of the assays) with a prevalence >5% in the dataset

Reactivity analysis updates are published as BioFire COVID-19 Test SARS-CoV-2 Reactivity Technical Note at https://www.biofiredefense.com/product-support/filmarray-support/

CONCLUSIONS

- BioFire COVID-19 Test 2 is a robust, sensitive, and specific multiplexed in vitro diagnostic device for the detection of SARS-CoV-2 RNA from NPS specimens in transport medium.
- Multiple assays within the test continue to ensure reactivity of BioFire COVID-19 Test 2 to evolving SARS-CoV-2 variants throughout the pandemic.

BIOFIRE DEFENSE

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