November 1, 2021

BioFire Defense, LLC
Cynthia Phillips
VP of Regulatory, Quality, and Clinical Affairs
79 W 4500 S, Suite 14
Salt Lake City, Utah 84107

Re: K211079
Trade/Device Name: BioFire COVID-19 Test 2
Regulation Number: 21 CFR 866.3981
Regulation Name: Device to detect and identify nucleic acid targets in respiratory specimens from microbial agents that cause the SARS-CoV-2 respiratory infection and other microbial agents when in a multi-target test
Regulatory Class: Class II
Product Code: QQX
Dated: April 8, 2021
Received: April 12, 2021

Dear Cynthia Phillips:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal

statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Kristian Roth, Ph.D.
Deputy Director
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K211079

Device Name
BioFire COVID-19 Test 2

Indications for Use *(Describe)*
The BioFire COVID-19 Test 2 is a qualitative nested multiplexed RT-PCR in vitro diagnostic test intended for use with the BioFire FilmArray 2.0 and BioFire FilmArray Torch Systems. The BioFire COVID-19 Test 2 detects nucleic acids from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal swabs (NPS) from symptomatic individuals suspected of COVID-19 by their healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in NPS specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out co-infection with other pathogens.

Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities. The BioFire COVID-19 Test 2 is intended for use by trained medical and laboratory professionals in a laboratory setting or under the supervision of a trained laboratory professional.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

I. Submitter

BioFire Defense, LLC
Salt Lake City, UT 84107
Phone: (801) 262-3592

Contact Person: Cynthia L. Phillips
Date Prepared: 2021-Apr-08

II. Device

Name of Device: BioFire® COVID-19 Test 2
Common or Usual Name: Same
Classification Name: Multi-Target Respiratory Specimen Nucleic Acid Test Including SARS-CoV-2 and Other Microbial Agents.
Regulatory Class: Class II (Special Controls)
Regulation: 21 CFR 866.3981
Panel: Microbiology – 83
Product Code: QQX

III. Predicate Device

BioFire® Respiratory Panel 2.1 (RP2.1) (BioFire Diagnostics, LLC) [DEN200031]
This predicate has not been subject to a design-related recall.

IV. Device Description

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive-sense, single-stranded RNA betacoronavirus and is the etiological agent of Coronavirus Disease 2019 (COVID-19).[1,2] The disease is primarily characterized by shortness of breath, fever or chills, cough, fatigue, and muscle or body aches.[2,3] Older adults or people with underlying medical conditions may be at higher risk for developing more severe cases which can be fatal.[2,3] The virus is thought to be of zoonotic origin and is highly transmissible through the inhalation of respiratory droplets.[1,3,4]

The BioFire COVID-19 Test 2 is a multiplexed nucleic acid-based test designed to be used with BioFire® FilmArray® Systems (BioFire® FilmArray® 2.0 or BioFire® FilmArray® Torch). The BioFire COVID-19 Test 2 consists of the BioFire COVID-19 Test 2 pouch which contains freeze-dried reagents to perform nucleic acid purification and nested, multiplexed polymerase chain reaction (PCR) with DNA melt analysis. The BioFire COVID-19 Test 2 conducts three independent tests for the detection of SARS-CoV-2 RNA in nasopharyngeal swabs (NPS) eluted in transport medium or saline. Results from the BioFire COVID-19 Test 2 are available in about 45 minutes.

A test is initiated by loading Hydration Solution into one port of the pouch and a NPS specimen mixed with the provided Sample Buffer into the other port of the pouch. The pouch contains all the reagents required for specimen testing and analysis in a freeze-dried format; the addition of Hydration Solution and the Sample Buffer rehydrates the reagents. After the pouch is prepared, the FilmArray Software on the FilmArray System guides the user through the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample identification, selecting the appropriate protocol, and initiating the run on the FilmArray System.

The FilmArray instruments contain a coordinated system of inflatable bladders and seal points, which act on the pouch to control the movement of liquid between the pouch blisters. When a bladder is inflated over a reagent blister, it forces liquid from the blister into connecting channels. Alternatively, when a seal is placed over a connecting channel it acts as a valve to open or close a channel. In addition, electronically controlled pneumatic pistons are positioned over multiple plungers to deliver the rehydrated reagents into the blisters at the appropriate times. Two Peltier devices control heating and cooling of the pouch to drive the PCR reactions and subsequent melt.

Nucleic acid extraction occurs within the FilmArray pouch using mechanical and chemical lysis followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, a nested multiplexed PCR is executed in two stages. During the first stage, a single, large volume, multiplexed reverse transcription PCR (rt-PCR) reaction is performed. The products from first stage PCR are then diluted and combined with a fresh, primer-free master mix and a fluorescent double stranded DNA binding dye (LC Green® Plus, BioFire Defense, LLC). The solution is then distributed to each well of the array. Array wells contain sets of primers designed specifically to amplify sequences internal to the PCR products generated during the first stage PCR reaction. The 2nd stage PCR and melt, or nested PCR, is performed in each well of the array. At the conclusion of the 2nd stage PCR, the array is interrogated by melt curve analysis for the detection of signature amplicons denoting the presence of specific targets. A digital camera placed in front of the array captures fluorescent images of the PCR2 reactions The FilmArray software automatically analyzes the results of each DNA melt curve and the results of the internal pouch controls to provide a final test interpretation.

Materials provided in each BioFire COVID-19 Test 2 Kit:

- Individually packaged BioFire COVID-19 Test 2 Pouches
- Single-use (1.0 mL) Sample Buffer Tubes
- Single-use pre-filled (1.5 mL) Hydration Injection Vials (blue)
- Individually packaged Sample Injection Vials (red)
- Individually packaged Transfer Pipettes

Materials required but not provided:

- 10% bleach solution
- FilmArray system including:
    - o FilmArray 2.0 instruments or FilmArray Torch modules and accompanying software
    - o FilmArray Pouch Loading Station

V. Intended Use

The BioFire® COVID-19 Test 2 is a qualitative nested multiplexed RT-PCR in vitro diagnostic test intended for use with the BioFire® FilmArray® 2.0 and BioFire® FilmArray® Torch Systems. The BioFire COVID-19 Test 2 detects nucleic acids from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal swabs (NPS) from symptomatic individuals suspected of COVID-19 by their healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in NPS specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out co-infection with other pathogens.

Results are meant to be used in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the relevant public health authorities. The BioFire COVID-19 Test 2 is intended for use by trained medical and laboratory professionals in a laboratory setting or under the supervision of a trained laboratory professional.

For In Vitro Diagnostic Use.

VI. Substantial Equivalence

The BioFire COVID-19 Test 2 and the BioFire Respiratory Panel 2.1 (RP2.1) are substantially equivalent. The BioFire COVID-19 Test 2 is essentially a BioFire RP2.1 pouch which has been modified to detect only SARS-CoV-2 RNA targets. The instrument platforms, sample type, chemistry, and protocols for the BioFire COVID-19 Test 2 are identical to those of the BioFire RP2.1. The BioFire RP2.1 was granted clearance on March 17, 2021 and determined to be a Class II device [DEN200031].

Table 1 compares the BioFire COVID-19 Test 2 to the BioFire Respiratory Panel 2.1 and outlines the similarities and differences between the two tests.

Table 1. Comparison of the BioFire COVID-19 Test 2 and the BioFire Respiratory Panel 2.1

Element	New Device: BioFire COVID-19 Test 2	Predicate Device: BioFire Respiratory Panel 2.1
Specimen Types	Nasopharyngeal swabs eluted in transport medium or saline	Same

Element	New Device: BioFire COVID-19 Test 2	Predicate Device: BioFire Respiratory Panel 2.1
Viruses/Organisms Detected	SARS-CoV-2 (multiple targets independent of BioFire RP2.1)	SARS-CoV-2 **AND** adenovirus, coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, human metapneumovirus, human rhinovirus/enterovirus, influenza A (including subtypes H1, H3, and H1-2009), influenza B, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, respiratory syncytial virus, *Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*
Analyte	RNA	DNA/RNA
Technological Principles	Nested multiplex RT-PCR followed by high resolution melting analysis to confirm identity of amplified product	Same
Instrumentation	FilmArray 2.0 or FilmArray Torch	Same
Time to Result	About 45 minutes	Same
Reagent Storage	Room temperature	Same
Test Interpretation	Automated test interpretation and report generation. User cannot access raw data.	Same
Controls	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis	Same
User Complexity	Moderate	Same

VII. Summary of Performance Data

Clinical Performance

The prospective clinical study evaluated the performance of the BioFire COVID-19 Test 2 with NPS specimens in transport medium. Specimens were residual after standard of care (SoC) testing for SARS-CoV-2 using a molecular test with Emergency Use Authorization (EUA). Specimens were de-identified and tested at three study sites in the United States over four months (July–October 2020) during the COVID-19 global pandemic.

This study was conducted as part of concurrent evaluation of the BioFire Diagnostics Respiratory Panel 2.1 (RP2.1). At the time of the study, BioFire RP2.1 was authorized under

EUA202392. In March 2021, BioFire RP2.1 was granted de novo classification under DEN200031. BioFire Diagnostics selected clinical sites, sponsored the study, and shared the collected data with BioFire Defense for secondary analysis.

A total of 534 specimens were assigned a study code number (SCN) and were enrolled in the study. Eleven (11/534) were excluded for various reasons. Seven (7/11) specimens failed inclusion criteria and were excluded: six did not meet storage requirements, and one failed to obtain a BioFire RP2.1 comparator result (a BioFire COVID-19 Test 2 was never attempted). Thus, 527 specimens met inclusion criteria and were available for testing on the BioFire COVID-19 Test 2. Four specimens (4/527) were later excluded from analyses due to inability to obtain a BioFire COVID-19 Test 2 result and not having sufficient specimen volume for retest.

The study included approximately equal numbers of females enrolled compared to males (48.4%, 253/523 and 51.1%, 267/523 respectively) (Table 2). Study enrollees were predominantly adults (only 10.5% (55/523) were 0-18 years).

Table 2. Overall Enrollment

		Overall
Sex	Female	**253 (48.4%)**
	Male	**267 (51.1%)**
	Unknown	**3 (0.6%)**
Age Range	0-18 years	**55 (10.5%)**
	19-40 years	**170 (32.5%)**
	41-60 years	**146 (27.9%)**
	61+ years	**152 (29.1%)**
Total		**523**

Specimens were tested on the BioFire COVID-19 Test 2 at clinical study sites. As a comparator for performance evaluation, specimens were also tested at clinical sites on BioFire RP2.1. A BioFire COVID-19 Test 2 result ('Detected' or 'Not Detected') was considered True Positive (TP) or True Negative (TN) only when it agreed with the BioFire RP2.1 comparator result. Positive Percent Agreement (PPA) or Sensitivity for each analyte was calculated as 100% × (TP / (TP + FN)). False Negative (FN) indicates that the BioFire COVID-19 Test 2 result was 'Not Detected', while the BioFire RP2.1 comparator result was positive. Negative Percent Agreement (NPA) or Specificity was calculated as 100% × (TN / (TN + FP)). False Positive (FP) indicates that the BioFire COVID-19 Test 2 result was 'Detected', but the BioFire RP2.1 comparator result was negative. The exact binomial two-sided 95% confidence interval was calculated. The prospective clinical study results are summarized in Table 3.

Table 3. BioFire COVID-19 Test 2 Clinical Performance Summary

Positive Agreement (PPA)				Negative Agreement (NPA)			
TP	**FN**	**%**	**95% CI**	**TN**	**FP**	**%**	**95% CI**
68	1[a]	98.6%	92.2-99.7%	452	2[b]	99.6%	98.4-99.9%

[a] The FN specimen was negative for SARS-CoV-2 by SoC and Central Reference Lab (CRL) testing.
[b] Evidence of SARS-CoV-2 was found in one FP by SoC and CRL testing.

Select Analytical Studies

Limit of Detection

The BioFire COVID-19 Test 2 limit of detection (LoD) was determined using contrived samples containing known concentrations of inactivated or infectious SARS-CoV-2 material. The LoD concentration was first estimated based on results of serial dilutions spanning concentrations bracketing the anticipated LoD concentration. Additional dilutions were tested, if needed, to reach a concentration at which loss of detection could be observed.

The LoD was then confirmed by testing 20 replicates at the estimated LoD concentration. Contrived specimens for LoD confirmation were prepared with SARS-CoV-2 at known concentrations in NPS eluted in transport medium. The required criteria for confirmation of LoD was a detection rate of at least 95% at the LoD concentration (≥ 19/20). Results are shown in Table 4.

Table 4. Summary of LoD Results for the BioFire COVID-19 Test 2

Virus	LoD Concentration	Detection Rate
SARS-CoV-2 USA-WA1/2020 (infectious culture; WRCEVA)[a]	3.3E+02 GC/mL (2.2E-02 $TCID_{50}$/mL)	20/20 (100%)
SARS-CoV-2 USA-WA1/2020 (heat-inactivated; BEI NR-52286)[b]	3.3E+02 GE/mL (4.3E-02 $TCID_{50}$/mL)	20/20 (100%)

[a] Obtained for culture in a biosafety level 3 laboratory from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA). Concentration determined by quantitative real-time PCR as described on the World Health Organization website: https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf

[b] Concentration determined by digital droplet PCR as indicated on the Certificate of Analysis provided by BEI Resources. $TCID_{50}$/mL was determined prior to inactivation.

NPS in Saline Sensitivity Validation

Sensitivity of the BioFire COVID-19 Test 2 when testing NPS collected in saline was evaluated by confirming the reliable detection (≥ 95%) of SARS-CoV-2 at 1× the LoD. Twenty (20) samples were individually contrived by spiking inactivated SARS-CoV-2 from the USA-WA1/2020 isolate (BEI/NR-52286) into NPS specimens that had been collected in saline. Each replicate was contrived at a concentration of 3.3E+02 GE/mL (1× LoD). Results, shown in Table 5, demonstrated equivalent sensitivity to samples prepared with NPS in transport medium.

Table 5. Summary of NPS in Saline Sensitivity Testing

Clinical Matrix	Test Concentration	Detection Rate
NPS in Saline	3.3E+02 GE/mL (1× LoD)	20/20 (100%)

FDA SARS-CoV-2 Reference Panel

SARS-CoV-2 sensitivity and MERS-CoV cross-reactivity were evaluated using the FDA SARS-CoV-2 Reference Panel according to the standard protocol provided by the FDA. The evaluation was performed using the supplied reference material and blinded samples. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The product LoD when using the FDA Reference Panel is presented in Table 6. No cross-reactivity with MERS-CoV was reported.

Table 6. Summary of FDA SARS-CoV-2 Reference Panel Testing

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	NPS in transport medium	5.4E+03 NDU/mL[1]	N/A[2]
MERS-CoV		N/A[2]	ND[3]

[1] NDU: Nucleic acid amplification test (NAAT) Detectable Units
[2] N/A: Not applicable
[3] ND: Not detected

Inclusivity (Reactivity)

Five variants of infectious SARS-CoV-2 were spiked into pooled NPS specimens in triplicate. Alignments of these SARS-CoV-2 variant sequences did not reveal any mismatches within the assay primer binding regions; therefore, selection of the variants was based on geographic location. Because the quantification method differed from the method used to quantify virus for LoD testing, the evaluation was carried out relative to the SARS-CoV-2 USA-WA1/2020 reference strain. The testing concentration of the reference strain was determined to be ~1× LoD. Results for inclusivity testing are summarized in Table 7. All four SARS-CoV-2 variants were detected within 3-fold the concentration of the USA-WA1/2020 reference variant. Mean melting temperature (Tm) values for each of the SARS-CoV-2 assays when testing the variants were within 0.5 °C of values obtained with the USA-WA1/2020 reference variant.

Table 7. Summary of Inclusivity Results for the BioFire COVID-19 Test 2

SARS-CoV-2 Variant (Source / ID)	SARS-CoV-2 Detection Rate	Concentration Detected	
		(GC/mL)	PFU/mL
USA-WA1/2020 (BEI / NR-52281)	3/3	9.9E+02	1.3E-02
Chile/Santiago_op4d1/2020 (BEI / NR-52439)	3/3	9.9E+02	6.3E-02
Hong Kong/VM20001061/2020 (BEI / NR-52282)	3/3	9.9E+02	1.5E-02
Italy-INMI1 (BEI / NR-52284)	3/3	9.9E+02	7.6E-02
New York-PV08410/2020	3/3	3.3E+03	2.4E-02

SARS-CoV-2 Variant (Source / ID)	SARS-CoV-2 Detection Rate	Concentration Detected	
		(GC/mL)	PFU/mL
(BEI / NR-53514)			

An in-silico analysis was also performed using publicly available SARS-CoV-2 sequences. At this time BioFire Defense predicts all strains of SARS-CoV-2 will be positively identified even if sensitivity to one or two assays is reduced. BioFire Defense will continue monitoring emerging SARS-CoV-2 strains and sequence variants and assessing any predicted changes in BioFire COVID-19 Test 2 performance.

Exclusivity (Specificity)

The potential for non-specific amplification and detection by the BioFire COVID-19 Test 2 was evaluated by challenging the test with a panel of 77 viruses and organisms at high concentrations in sterile saline. The panel of viruses and organisms included normal respiratory flora and pathogens that may be present in NPS specimens, as well as SARS-CoV-2 related viruses. A list of viruses and organisms tested is shown in Table 8. No assay cross-reactivity with any of the tested viruses/organisms was observed.

Table 8. List of Viruses/Organisms Tested for Exclusivity on the BioFire COVID-19 Test 2

Organism/Virus		
Viruses (SARS-CoV-2 Related)		
Human coronavirus 229E	Human coronavirus NL63	Middle East Respiratory Syndrome coronavirus (MERS-CoV)
Human coronavirus HKU1	Human coronavirus OC43	Severe Acute Respiratory Syndrome coronavirus (SARS-CoV)
Viruses		
Adenovirus 1 (species C)	Enterovirus species D (68)	Mumps virus
Adenovirus 4 (species E)	Epstein-Barr virus (B95-8)	Parainfluenza virus 1
Adenovirus 7 (species B)	Herpes simplex virus	Parainfluenza virus 2
Bocavirus	Human Metapneumovirus	Parainfluenza virus 3
Cytomegalovirus	Influenza A subtype H1	Parainfluenza virus 4
Enterovirus species A (EV71)	Influenza A subtype H3	Respiratory syncytial virus
Enterovirus species B (Echovirus 6)	Influenza B	Rhinovirus
Enterovirus species C (Coxsackievirus A17)	Measles virus	
Bacteria		

Organism/Virus		
Acinetobacter calcoaceticus	*Klebsiella aerogenes* (*Enterobacter aerogenes*)	*Neisseria elongate*
Bordetella avium	*Klebsiella oxytoca*	*Neisseria gonorrhoeae*
Bordetella bronchiseptica	*Klebsiella pneumoniae*	*Neisseria meningitidis*
Bordetella hinzii	*Lactobacillus acidophilus*	*Proteus mirabilis*
Bordetella holmesii	*Lactobacillus plantarum*	*Pseudomonas aeruginosa*
Bordetella parapertussis	*Legionella feeleii*	*Serratia marcescens*
Bordetella pertussis	*Legionella longbeacheae*	*Staphylococcus aureus*
Chlamydia pneumoniae	*Legionella pneumophila*	*Staphylococcus epidermidis*
Chlamydia trachomatis	*Moraxella catarrhalis*	*Stenotrophomonas maltophilia*
Corynebacterium diptheriae	*Mycobacterium tuberculosis*	*Streptococcus agalactiae*
Escherichia coli	*Mycoplasma genitalium*	*Streptococcus pneumoniae*
Fluoribacter bozemanae (*Legionella bozemanii*)	*Mycoplasma hominis*	*Streptococcus pyogenes*
Fluoribacter dumoffii (*Legionella dumoffii*)	*Mycoplasma orale*	*Streptococcus salivarius*
Haemophilus influenzae	*Mycoplasma pneumoniae*	*Tatlockia micdadei* (*Legionella micdadei*) *Ureaplasma urealyticum*
Fungi		
Aspergillus flavus	*Candida albicans*	*Pneumocystis jirovecii*
Aspergillus fumigatus	*Cryptococcus neoformans*	

An in-silico analysis was also performed using an alignment of 1,124 publicly available *Coronaviridae* sequences. Two non-SARS-CoV-2 accessions were predicted to be cross-reactive with the BioFire COVID-19 Test 2: bat coronavirus RaTG13 (MN996532) and pangolin coronavirus isolate MP789 (MT084071). Neither of these viruses are expected to be present within human NPS specimens. However, if present, the cross-reactive product(s) may be detected as SARS-CoV-2.

Interference

For this study, potentially interfering substances were selected based upon FDA recommended interferents specific to the respiratory sample type. Potential interference was evaluated by comparing BioFire COVID-19 Test 2 results from samples containing SARS-CoV-2 analyte at 1× LoD to results from samples with the same analyte composition but including the test substance. Potential effects of the substance on the BioFire COVID-19 Test 2 control assays and analyte detection were evaluated. Table 9 provides a list of the potentially interfering substances evaluated during this study and the observed results of testing in the presence of the substances. None of the substances tested were determined to be inhibitory to the BioFire COVID-19 Test 2.

Table 9. Results of Potentially Interfering Substances Testing on the BioFire COVID-19 Test 2

Substance	Specific Active Ingredient	Test Concentration
Toothpaste (Colgate Total)	Stannous fluoride 0.454%	2% v/v
Tobacco (Camel Snus)	Tobacco	10 mg/mL
Oral Rinse (Listerine)	Eucalyptol 0.092% Menthol 0.042% Methyl Salicylate 0.060% Thymol 0.064%	1% v/v
Throat lozenges (Cepacol)	Benzocaine 7.5 mg Dextromethorphan HBr 5 mg	2.2 mg/mL
Oral anesthetic and analgesic (Mouth Sore Relief)	Benzocaine 20%	1% v/v
Cough Drops	Menthol 5.4 mg	2.2 mg/mL
Cleanser (Dye-Free Antiseptic Cleanser)	Chlorhexidine Gluconate 4%	1% v/v
Nicotine	Nicotine	10 mg/mL
Mucin	Bovine submaxillary gland, type I-S Sigma M3895	5 mg/mL
Blood (human)	Human DNA	5% v/v
Leukocytes	Human DNA	1% v/v
Nasal spray (Wal-Four Nasal Spray)	Phenylephrine hydrochloride 1%	10% v/v
Afrin Nasal Spray	Oxymetazoline hydrochloride 0.05%	10% v/v
Saline Nasal Spray	NaCl 0.65% with preservatives (Phenylcarbinol, Benzalkonium Chloride)	10% v/v
Nasal corticosteroids	Beclomethasone	2 mg/mL
	Dexamethasone	1.5 mg/mL
	Flunisolide	2 mg/mL
	Triamcinolone	5 mg/mL
	Mometasone	1 mg/mL
Budesonide Nasal Spray	Budesonide 32 mcg/ spray	1% v/v
Allergy Nasal Spray	Fluticasone 50 mcg/ spray	1% v/v
Nasal gel (Zicam)	Luffa opperculata 4x Galphimia glauca 4x Sabadilla 4x	1% v/v
Sulfur	Sulfur	0.17 mg/mL
Allergy Relief (RhinAllergy)	Histaminum hydrochloricum 9C HPUS	10 mg/mL
Anti-viral drugs	Zanamivir	5.5 mg/mL
Antibiotic, Nasal ointment	Mupirocin	3.3 mg/mL
Antibacterial, systemic	Tobramycin	4 µg/mL
Transport Media	Remel M4 (R12503)	100%
	Remel M4RT (R12591)	100%
	Copan UTM-RT (UTM 330C)	100%
	PrimeStore MTM (MTM-LH102)	100%

Substance	Specific Active Ingredient	Test Concentration
	Merit Medical Cultura Media	100%
	Neuronics VTM	100%
	Azer UTM	100%
	Bartels FlexTrans	100%
	S2 VTM	100%
Diluents	Ethanol	10% v/v
	DMSO	10% v/v
	Methanol	10% v/v
	Chloroform	10% v/v
	DMF	10% v/v

Reproducibility

Assay reproducibility was evaluated by preparing three samples using pooled negative clinical NPS matrix. Two samples were spiked with SARS-CoV-2 at either moderate positive (3× LoD) or low positive (1× LoD) concentrations; a third sample was not spiked (negative sample). Six replicates of each sample were tested at three locations over five different days, providing a total of 90 replicate test results per sample. Reproducibility was evaluated using both FilmArray 2.0 instruments and FilmArray Torch modules. BioFire COVID-19 Test pouch reagent lots were rotated daily. In total, 270 valid test results were obtained.

The primary assessment of reproducibility was based on a comparison of the observed test results ('Detected'/ 'Not Detected') to the expected test results. The detection rate and percent agreement between observed and expected test results are shown in Table 10; the expected percent agreement was ≥ 95%. Based on the percent agreement between observed and expected test results and the variability in Tm (standard deviations ≤0.3 °C, CV ≤0.4%), the reproducibility data demonstrates that the BioFire COVID-19 Test 2 can provide accurate and highly reproducible test results in the context of multiple variables that may be expected in a clinical testing environment.

Table 10. Summary of BioFire COVID-19 Test 2 Reproducibility Results

Analyte (Source / ID)	Test Concentration ×LoD (GE/mL)	Expected Result	Detection Rate (n/N) (% Agreement with Expected Result)			
			Site 1	Site 2	Site 3	All Sites [95% Conf. Interval]
SARS-CoV-2 USA-WA1/2020 (BEI / NR-52286)	Moderate Positive 3× LoD (9.9E+02)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%) [95.9-100%]
	Low Positive 1× LoD (3.3E+02)	Detected	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%) [95.9-100%]

	Negative (No Analyte)	Not Detected	30/30 (100%)	28/30 (93.3%)	29/30 (96.7%)	87/90 [a] (96.7%) [90.7-98.9%]

[a] Clinical NPS specimens in transport medium were collected during the COVID-19 outbreak and therefore SARS-CoV-2 at levels below LoD may have gone undetected during characterization of the pooled sample matrix.

Specimen Storage

Ten samples were contrived by spiking pooled clinical NPS specimens with SARS-CoV-2 at three concentrations relative to the limit of detection (LoD) (3× LoD, 5× LoD, and 10× LoD). Each contrived specimen was tested immediately as a control, then aliquoted and stored under six different storage conditions. Storage conditions were selected based on the desired claims for specimen storage and included one additional time point beyond the desired claim. A summary of detection rates under each storage condition is shown in Table 11. These data support the claimed storage conditions for NPS specimens as up to 4 hours at 15-30˚C, up to 3 days at 2-8˚C, and up to 30 days at ≤ -15˚C.

Table 11. Summary of BioFire COVID-19 Test 2 Specimen Storage Testing

Test Concentration	No Storage Control	Ambient (30˚C)		Refrigerated (2-8˚C)		Frozen (≤ -15˚C)	
		4 Hrs.	6 Hrs.	3 Days	6 Days	30 Days	34 Days
	Detection Rate (%)	Detection Rate (%)	Detection Rate (%)	Detection Rate (%)	Detection Rate (%)	Detection Rate (%)	Detection Rate (%)
9.9E+02 GE/mL (3× LoD)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
1.6E+03 GE/mL (5× LoD)	2/2 (100%)	2/2 (100%)	2/2 (100%)	2/2 (100%)	2/2 (100%)	2/2 (100%)	2/2 (100%)
3.3E+03 GE/mL (10× LoD)	2/2 (100%)	2/2 (100%)	2/2 (100%)	2/2 (100%)	2/2 (100%)	2/2 (100%)	2/2 (100%)

VIII. References

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