March 26, 2024

BioFire Diagnostics, LLC
Kevin Bourzac
VP, Regulatory and Clinical Affairs-Molecular
515 Colorow Drive
Salt Lake City, Utah 84108

Re: K232954
Trade/Device Name: BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel
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: QOF, OEM, OOU, OTG, OZE, OZX, OZY, OZZ, OCC, NSU, PGX
Dated: September 20, 2023
Received: September 21, 2023

Dear Kevin Bourzac:

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 (the 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 available 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.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device"

(https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

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 Part 803) for devices or postmarketing safety reporting (21 CFR Part 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 Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 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,

Joseph Briggs -S

Joseph Briggs
Deputy Branch Chief
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K232954

Device Name
BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel

Indications for Use *(Describe)*

The BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel is a multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE® SPOTFIRE® System for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab (NPS) specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19; (Respiratory menu) or in throat swab (TS) specimens from individuals with signs and symptoms of pharyngitis (Sore Throat menu).

The following organism types and subtypes are identified and differentiated using the SPOTFIRE R/ST Panel:

Respiratory Menu	Sore Throat Menu
Viruses	Viruses
Adenovirus	Adenovirus
Coronavirus SARS-CoV-2	Coronavirus (seasonal)
Coronavirus (seasonal)	Human metapneumovirus
Human metapneumovirus	Human rhinovirus/enterovirus
Human rhinovirus/enterovirus	Influenza A virus
Influenza A virus	Influenza A virus/H1-2009
Influenza A virus/H1-2009	Influenza A virus/H3
Influenza A virus/ H3	Influenza B virus
Influenza B virus	Parainfluenza virus
Parainfluenza virus	Respiratory syncytial virus
Respiratory syncytial virus	
Bacteria	Bacteria
Bordetella parapertussis	Chlamydia pneumoniae
Bordetella pertussis	Mycoplasma pneumoniae
Chlamydia pneumoniae	Streptococcus dysgalactiae (Group C/G Strep)
Mycoplasma pneumoniae	Streptococcus pyogenes (Group A Strep)

Nucleic acids from the viral and bacterial organisms identified by this test are generally detectable in NPS/TS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of respiratory infection and/or pharyngitis are indicative of the presence of the identified microorganism and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Negative results in the setting of a respiratory illness and/or pharyngitis may be due to infection with pathogens that are not detected by this test, or a respiratory tract infection that may not be detected by an NPS or TS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the SPOTFIRE R/ST Panel may not be the definite cause of disease.

Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection and/or pharyngitis.

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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BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel for use on the BIOFIRE® SPOTFIRE® System

510(k) Summary
BioFire Diagnostics, LLC

Introduction:

The purpose of this Dual 510(k) and CLIA Waiver submission is to obtain clearance for the BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel (SPOTFIRE R/ST Panel).

The SPOTFIRE R/ST Panel simultaneously identifies 15 different respiratory viral and bacterial pathogens in nasopharyngeal swabs (NPS) or 14 viral and bacterial pharyngitis pathogens in throat swabs (TS) from individuals with signs and symptoms of respiratory tract infections or pharyngitis, respectively. Users have the option of choosing a Respiratory Menu or Sore Throat Menu, depending on the sample type. The SPOTFIRE R/ST Panel is compatible with the BIOFIRE® SPOTFIRE® System (SPOTFIRE System), a polymerase chain reaction (PCR)-based in vitro diagnostic system for infectious disease testing (K213954/ CW210006).

According to the requirements of 21 CFR 807.92, the information included with this submission provides sufficient detail to understand the basis for a determination of substantial equivalence.

Submitted by:

BioFire Diagnostics, LLC
515 Colorow Drive
Salt Lake City, UT 84108

Contact: Kevin Bourzac, Ph.D.
Telephone: 801-736-6354, ext. 1358
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Email: Kevin.Bourzac@biomerieux.com

Date submitted: March 05, 2024

Device Name and Classification:

Trade name: BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel

Primary Regulation Number for device classification: 21 CFR 866.3981

Classification Name: Multi-Target Respiratory Specimen Nucleic Acid Test Including SARS-CoV-2 And Other Microbial Agents

Additional Regulation Numbers: 21 CFR 866.2680, 21 CFR 866.3980, 21 CFR 862.2570

Predicate Device:

K213954/CW210006 – BIOFIRE® SPOTFIRE® Respiratory (R) Panel

Intended Use:

The BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel is a multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE® SPOTFIRE® System for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab (NPS) specimens obtained from **individuals with signs and symptoms of respiratory tract infection**, **including COVID-19**; (**Respiratory menu**) or in throat swab (TS) specimens from **individuals with signs and symptoms of pharyngitis** (**Sore Throat menu**).

The following organism types and subtypes are identified and differentiated using the SPOTFIRE R/ST Panel:

Respiratory Menu	**Sore Throat Menu**
Viruses	**Viruses**
Adenovirus	Adenovirus
Coronavirus SARS-CoV-2	Coronavirus (seasonal)
Coronavirus (seasonal)	Human metapneumovirus
Human metapneumovirus	Human rhinovirus/enterovirus
Human rhinovirus/enterovirus	Influenza A virus
Influenza A virus	Influenza A virus/H1-2009
Influenza A virus/H1-2009	Influenza A virus/H3
Influenza A virus/H3	Influenza B virus
Influenza B virus	Parainfluenza virus
Parainfluenza virus	Respiratory syncytial virus
Respiratory syncytial virus	
Bacteria	**Bacteria**
Bordetella parapertussis	*Chlamydia pneumoniae*
Bordetella pertussis	*Mycoplasma pneumoniae*
Chlamydia pneumoniae	*Streptococcus dysgalactiae* (Group C/G Strep)
Mycoplasma pneumoniae	*Streptococcus pyogenes* (Group A Strep)

Nucleic acids from the viral and bacterial organisms identified by this test are generally detectable in NPS/TS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of respiratory infection and/or pharyngitis are indicative of the presence of the identified microorganism and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Negative results in the setting of a respiratory illness and/or pharyngitis may be due to infection with pathogens that are not detected by this test, or a respiratory tract infection that may not be detected by an NPS or TS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the SPOTFIRE R/ST Panel may not be the definite cause of disease.

Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection and/or pharyngitis.

Device Description:

The SPOTFIRE R/ST Panel simultaneously identifies 15 different respiratory viral and bacterial pathogens in nasopharyngeal swabs (NPS) or 14 viral and bacterial pharyngitis pathogens in throat swabs (TS) from individuals with signs and symptoms of respiratory tract infections or pharyngitis, respectively, (see Intended Use:). The SPOTFIRE R/ST Panel is compatible with the SPOTFIRE System, a polymerase chain reaction (PCR)-based in vitro diagnostic system for infectious disease testing. The SPOTFIRE System Software executes the SPOTFIRE R/ST Panel test and interprets and reports the test results. The SPOTFIRE R/ST Panel was designed to be used in CLIA-waived environments. A test is initiated by loading Hydration Solution into one port of the SPOTFIRE R/ST Panel pouch and NPS or TS specimen, mixed with the provided Sample Buffer, into the other port of the SPOTFIRE R/ST Panel pouch and placing it in the SPOTFIRE System. The pouch contains all of the reagents required for specimen testing and analysis in a freeze-dried format; the addition of Hydration Solution and Sample/Buffer Mix rehydrates the reagents. After the pouch is prepared, the SPOTFIRE System Software guides the user through the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample identification, and initiating the run.

The SPOTFIRE System contains coordinated systems 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 in order 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 the melt curve analysis.

Nucleic acid extraction occurs within the SPOTFIRE R/ST Panel pouch using mechanical and chemical lysis followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, the SPOTFIRE System performs a nested multiplex PCR that is executed in two stages. During the first stage, the SPOTFIRE System performs a single, large volume, highly multiplexed reverse transcription PCR (rt-PCR) reaction. 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 Diagnostics). 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, or nested PCR, is performed in singleplex fashion 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 2nd stage PCR captures fluorescent images of the PCR reactions and software interprets the data.

The SPOTFIRE System Software automatically interprets the results of each DNA melt curve analysis and combines the data with the results of the internal pouch controls to provide a test result for each organism on the SPOTFIRE R/ST Panel.

Substantial Equivalence:

The SPOTFIRE R/ST Panel is substantially equivalent to the SPOTFIRE R Panel (K213954/CW210006), which was cleared on February 03, 2023, and determined to be a Class II device under the classification code 21 CFR 866.3981.

A table comparing the SPOTFIRE R/ST Panel to the SPOTFIRE R Panel is provided in Table 1.

Table 1. Similarities and differences between the SPOTFIRE R Panel and the SPOTFIRE R/ST Panel

Element	Predicate: SPOTFIRE R Panel (K213954/CW210006)	New Device: SPOTFIRE R/ST Panel
Intended Use	The BIOFIRE® SPOTFIRE® Respiratory (R) Panel (SPOTFIRE R Panel) is a multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE® SPOTFIRE® System for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab (NPS) specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19. The following organism types and subtypes are identified and differentiated using the SPOTFIRE R Panel: <u>Viruses</u> Adenovirus Coronavirus (seasonal) Coronavirus SARS-CoV-2 Human metapneumovirus Human rhinovirus/enterovirus Influenza A virus Influenza A virus A/H1-2009 Influenza A virus A/H3 Influenza B virus Parainfluenza virus Respiratory syncytial virus <u>Bacteria</u> *Bordetella parapertussis* *Bordetella pertussis* *Chlamydia pneumoniae* *Mycoplasma pneumoniae* Nucleic acids from the viral and bacterial organisms identified by this test are generally detectable in NPS	The BIOFIRE® SPOTFIRE® Respiratory/Sore Throat (R/ST) Panel is a multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE SPOTFIRE System for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab (NPS) specimens obtained from **individuals with signs and symptoms of respiratory tract infection**, **including COVID-19**; (**Respiratory menu**) or in throat swab (TS) specimens from **individuals with signs and symptoms of pharyngitis** (**Sore Throat menu**). The following organism types and subtypes are identified and differentiated using the SPOTFIRE R/ST Panel: **<u>Respiratory Menu</u>** <u>Viruses</u> Adenovirus Coronavirus SARS-CoV-2 Coronavirus (seasonal) Human metapneumovirus Human rhinovirus/enterovirus Influenza A virus Influenza A virus A/H1-2009 Influenza A virus A/H3 Influenza B virus Parainfluenza virus Respiratory syncytial virus <u>Bacteria</u> *Bordetella parapertussis* *Bordetella pertussis* *Chlamydia pneumoniae* *Mycoplasma pneumoniae*

	specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection are indicative of the presence of the identified microorganism and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the SPOTFIRE R Panel may not be the definite cause of disease. Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.	**Sore Throat Menu** Viruses Adenovirus Coronavirus (seasonal) Human metapneumovirus Human rhinovirus/enterovirus Influenza A virus Influenza A virus A/H1-2009 Influenza A virus A/H3 Influenza B virus Parainfluenza virus Respiratory syncytial virus Bacteria *Chlamydia pneumoniae* *Mycoplasma pneumoniae* *Streptococcus dysgalactiae* (Group C/G Strep) *Streptococcus pyogenes* (Group A Strep) Nucleic acids from the viral and bacterial organisms identified by this test are generally detectable in NPS/TS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of respiratory infection and/or pharyngitis are indicative of the presence of the identified microorganism and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness and/or pharyngitis may be due to infection with pathogens that are not detected by this test, or a respiratory tract infection that may not be detected by an NPS or TS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the SPOTFIRE R/ST Panel may not be the definite cause of disease. Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection and/or pharyngitis.
Specimen Types	Nasopharyngeal swab in viral transport media	Nasopharyngeal swab in transport media or Throat swab in transport media

Organisms detected	**Viruses** Adenovirus Coronavirus (seasonal) Coronavirus SARS-CoV-2 Human metapneumovirus Human rhinovirus/enterovirus Influenza A virus Influenza A virus A/H1-2009 Influenza A virus A/H3 Influenza B virus Parainfluenza virus Respiratory syncytial virus **Bacteria** *Chlamydia pneumoniae* *Mycoplasma pneumoniae* *Bordetella parapertussis* *Bordetella pertussis*	Same, except: Additionally detects: *Streptococcus dysgalactiae* (Group C/G Strep) *Streptococcus pyogenes* (Group A Strep)
Analytes	DNA/RNA	Same
Technological Principles	Highly multiplexed nested nucleic acid amplification test with melt analysis	Same
Instrumentation	SPOTFIRE System	Same
Time to result	About 15 minutes	Same
Reagent Storage	Room Temperature	Same
Test Interpretation	Automated test interpretation and reporting. 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	Low (CLIA-waived)	Same
Panel Software Functions	Defines panel-specific parameters, instrument protocols and report requirements.	Same
	Analyzes processed image data (fluorescence and temperature data) and provides test results.	Same

Summary of Performance Data:

The performance data for the SPOTFIRE R/ST Panel is summarized in the *BIOFIRE SPOTFIRE Respiratory/Sore Throat Instructions for Use*. A summary of the R/ST Panel performance data is also provided below.

Clinical Performance

Table 2 and Table 3 provide a summary of the performance of each analyte from all studies performed for NPS and TS specimens, respectively.

Table 2. R/ST Panel Performance Summary for NPS Specimens (Respiratory Menu; as tested by intended users)

SPOTFIRE R/ST Panel R Menu Result	Study	Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Viruses							
Adenovirus	Prospective	32/33	97.0	84.7-99.5%	1058/1082	97.8	96.7-98.5%
	Archived	31/31	100	89.0-100%	439/453	96.9	94.9-98.2%
	Contrived	0/0	-	-	0/0	-	-
Coronavirus SARS-CoV-2	Prospective	71/73	97.3	90.5-99.2%	1031/1037	99.4	98.7-99.7%
	Archived	0/0	-	-	0/0	-	-
	Contrived	0/0	-	-	0/0	-	-
Coronavirus (seasonal)	Prospective	101/102	99.0	94.7-99.8%	1000/1013	98.7	97.8-99.2%
	Archived	95/96	99.0	94.3-99.8%	381/388	98.2	96.3-99.1%
	Contrived	0/0	-	-	0/0	-	-

SPOTFIRE R/ST Panel R Menu Result	Study	Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Human metapneumovirus	Prospective	1/1	100	-	1114/1114	100	99.7-100%
	Archived	32/33	97.0	84.7-99.5%	451/451	100	99.2-100%
	Contrived	0/0	-	-	0/0	-	-
Human rhinovirus/enterovirus	Prospective	345/348	99.1	97.5-99.7%	695/767	90.6	88.3-92.5%
	Archived	29/30	96.7	83.3-99.4%	439/454	96.7	94.6-98.0%
	Contrived	0/0	-	-	0/0	-	-
Influenza A virus	Prospective	0/0	-	-	1115/1115	100	99.7-100%
	Archived	58/59	98.3	91.0-99.7%	423/423	100	99.1-100%
	Contrived	0/0	-	-	0/0	-	-
Influenza A virus A/H1-2009	Prospective	0/0	-	-	1115/1115	100	99.7-100%
	Archived	31/32	96.9	84.3-99.4%	450/450	100	99.2-100%
	Contrived	0/0	-	-	0/0	-	-
Influenza A virus A/H3	Prospective	0/0	-	-	1115/1115	100	99.7-100%
	Archived	27/27	100	87.5-100%	455/455	100	99.2-100%
	Contrived	0/0	-	-	0/0	-	-
Influenza B virus	Prospective	0/0	-	-	1110/1110	100	99.7-100%
	Archived	30/30	100	88.6-100%	28/28	100	87.9-100%
	Contrived	0/0	-	-	0/0	-	-
Parainfluenza virus	Prospective	96/98	98.0	92.9-99.4%	1006/1017	98.9	98.1-99.4%
	Archived	116/118	98.3	94.0-99.5%	359/366	98.1	96.1-99.1%
	Contrived	0/0	-	-	0/0	-	-
Respiratory syncytial virus	Prospective	26/27	96.3	81.7-99.3%	1086/1088	99.8	99.3-100%
	Archived	37/37	100	90.6-100%	440/447	98.4	96.8-99.2%
	Contrived	0/0	-	-	0/0	-	-
Bacteria							
Bordetella parapertussis	Prospective	0/0	-	-	1110/1110	100	99.7-100%
	Archived	24/25	96.0	80.5-99.3%	33/33	100	89.6-100%
	Contrived	0/0	-	-	0/0	-	-
Bordetella pertussis	Prospective	0/0	-	-	1115/1115	100	99.7-100%
	Archived	27/28	96.4	82.3-99.4%	452/456	99.1	97.8-99.7%
	Contrived	0/0	-	-	0/0	-	-
Chlamydia pneumoniae	Prospective	0/0	-	-	1115/1115	100	99.7-100%
	Archived	30/30	100	88.6-100%	452/454	99.6	98.4-99.9%
	Contrived	0/0	-	-	0/0	-	-
Mycoplasma pneumoniae	Prospective	0/0	-	-	1115/1115	100	99.7-100%
	Archived	33/33	100	89.6-100%	446/451	98.9	97.4-99.5%
	Contrived	0/0	-	-	0/0	-	-

Table 3. R/ST Panel Performance Summary for TS Specimens (Sore Throat Menu; as tested by intended users)[a]

SPOTFIRE R/ST Panel ST Menu Result	Study	Sensitivity/PPA			Specificity/NPA		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Viruses							
Adenovirus	Prospective	60/65	92.3	83.2-96.7%	798/810	98.5	97.4-99.2%
	Archived	9/11	81.8	52.3-94.9%	44/48	91.7	80.4-96.7%
	Contrived	50/50	100	92.9-100%	380/381	99.7	98.5-100%
Coronavirus (seasonal)	Prospective	41/42	97.6	87.7-99.6%	826/833	99.2	98.3-99.6%
	Archived	12/12	100	75.8-100%	47/47	100	92.4-100%
	Contrived	129/130	99.2	95.8-99.9%	301/301	100	98.7-100%
Human metapneumovirus	Prospective	15/17	88.2	65.7-96.7%	858/858	100	99.6-100%
	Archived	4/4	100	51.0-100%	55/55	100	93.5-100%

SPOTFIRE R/ST Panel ST Menu Result	Study		Sensitivity/PPA			Specificity/NPA		
			TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
	Contrived		45/49	91.8	80.8-96.8%	382/382	100	99.0-100%
Human rhinovirus/enterovirus	Prospective		202/213	94.8	91.0-97.1%	619/662	93.5	91.4-95.1%
	Archived		2/2	100	34.2-100%	55/57	96.5	88.1-99.0%
	Contrived		0/0	-	-	0/0	-	-
Influenza A virus	Prospective		35/35	100	90.1-100%	840/840	100	99.5-100%
	Archived		11/11	100	74.1-100%	44/44	100	92.0-100%
	Contrived		93/93	100	96.0-100%	332/332	100	98.9-100%
Influenza A virus A/H1-2009	Prospective		12/12	100	75.8-100%	863/863	100	99.6-100%
	Archived		7/7	100	64.6-100%	48/48	100	92.6-100%
	Contrived		46/46	100	92.3-100%	379/379	100	99.0-100%
Influenza A virus A/H3	Prospective		23/23	100	85.7-100%	852/852	100	99.6-100%
	Archived		4/4	100	51.0-100%	51/51	100	93.0-100%
	Contrived		47/47	100	92.4-100%	378/378	100	99.0-100%
Influenza B virus	Prospective		4/4	100	51.0-100%	872/872	100	99.6-100%
	Archived		20/20	100	83.9-100%	0/0	-	-
	Contrived		47/49	95.9	86.3-98.9%	333/333	100	98.9-100%
Parainfluenza virus	Prospective		50/52	96.2	87.0-98.9%	821/823	99.8	99.1-99.9%
	Archived		1/1	100	-	58/58	100	93.8-100%
	Contrived		122/128	95.3	90.2-97.8%	303/303	100	98.7-100%
Respiratory syncytial virus	Prospective		21/24	87.5	69.0-95.7%	849/851	99.8	99.1-99.9%
	Archived		2/2	100	34.2-100%	56/57	98.2	90.7-99.7%
	Contrived		49/50	98.0	89.5-99.6%	381/381	100	99.0-100%
Bacteria								
Chlamydia pneumoniae	Prospective		0/0	-	-	875/875	100	99.6-100%
	Archived		2/2	100	34.2-100%	57/57	100	93.7-100%
	Contrived		50/50	100	92.9-100%	381/381	100	99.0-100%
Mycoplasma pneumoniae	Prospective		0/0	-	-	875/875	100	99.6-100%
	Archived		4/4	100	51.0-100%	55/55	100	93.5-100%
	Contrived		48/50	96.0	86.5-98.9%	381/381	100	99.0-100%
Streptococcus dysgalactiae (Group C/G Strep)	Prospective	PCR	30/30	100	88.6-100%	843/847	99.5	98.8-99.8%
		Culture	26/26	100	87.1-100%	837/843	99.3	98.5-99.7%
	Archived		0/0	-	-	0/0	-	-
	Contrived		0/0	-	-	0/0	-	-
Streptococcus pyogenes (Group A Strep)	Prospective	PCR	209/217	96.3	92.9-98.1%	654/660	99.1	98.0-99.6%
		Culture	174/177	98.3	95.1-99.4%	654/692	94.5	92.6-96.0%
	Archived		38/39	97.4	86.8-99.5%	10/10	100	72.2-100%
	Contrived		0/0	-	-	0/0	-	-

[a] The performance measures of sensitivity and specificity only refer to the Prospective and Archived *Streptococcus* analytes for which culture was used as the reference method. Performance measures of PPA and NPA refer to all other analytes, for which molecular assays or known specimen composition were used as comparator methods.

Analytical Performance Characteristics

Bench performance (analytical) testing for the SPOTFIRE R/ST Panel was designed to validate the performance of all analytes and to support testing of both sample types, NPS and TS specimens. Table 4 provides an overall summary of the analytical studies, their results, and conclusions.

Table 4. Summary of Bench Performance (Analytical) Testing for the SPOTFIRE R/ST Panel

Study	Description	Acceptance Criteria	Results	Conclusion
Sample Storage and Handling	The purpose of this study was to establish that accurate test results are observed for all SPOTFIRE R/ST Panel analytes, including sore throat-specific bacteria that had not been previously evaluated, when throat swab (TS) specimens in Amies media (TSa) are stored within the same range of storage conditions previously validated for nasopharyngeal swab (NPS) specimens in liquid media.	For the storage condition to be considered acceptable for each organism, 100% expected positive results were required to be observed in all samples tested. In addition, crossing point (Cp) values were evaluated for each relevant assay and trended across the conditions to assess analyte stability over time.	Positive results were observed in 100% of all TSa samples tested at all conditions evaluated for all SPOTFIRE R/ST Panel analytes.	The SPOTFIRE R/ST Panel provides accurate results when TS specimens are stored in Amies media for up to 4 hours at ambient temperature (15-25 °C), up to 3 days at refrigerated temperature (2-8 °C), and up to 30 days at frozen temperature (≤ -15 °C). Similar results were previously observed with NPS specimens stored in transport media.
Limit of Detection	The purpose of this study was to determine the Limit of Detection (LoD) for all analytes detected by the SPOTFIRE R/ST Panel in two common liquid media, Viral Transport Media (VTM) and Amies media. In addition, testing was performed to assess whether the presence of multiple organisms in a sample has an impact on the ability of the panel to detect low level analytes compared to samples containing only one organism.	The LoD for each SPOTFIRE R/ST Panel analyte was confirmed when positive results were reported in at least 95% (≥19/20) of replicates tested at the LoD (1× LoD), and fewer than 95% (≤18/20) of replicates tested at a concentration 10-fold below LoD (0.1× LoD). Equivalent detection of representative analytes in single analyte and multi-analyte samples was determined primarily based on concordance of positive or negative results at each test concentration.	The LoD concentrations for the SPOTFIRE R/ST Panel analytes were confirmed in viable or infectious units (e.g. TCID50/mL, CFU/mL, cells/mL, IFU/mL, CEID50/mL, CCU/mL) and/or nucleic acid copies/mL. The panel accurately detected viruses and bacteria in samples contrived in either VTM or Amies media containing one or multiple organisms.	The LoD was determined for each analyte detected by the SPOTFIRE R/ST Panel in VTM and/or Amies media, where appropriate, for the Respiratory and Sore Throat menus, respectively. The SPOTFIRE R/ST panel provides accurate detection results for all analytes in single or polymicrobial specimens when present at or above the LoD. No adverse effect on the analytical sensitivity of the SPOTFIRE R/ST Panel was observed when evaluating multi-analyte specimens.

Study	Description	Acceptance Criteria	Results	Conclusion
Analytical Reactivity (Inclusivity)	The purpose of this study was to evaluate the analytical reactivity (inclusivity) of the SPOTFIRE R/ST Panel assays.	The primary data evaluated for this study were the reported positive, negative, and uncertain results. The assay reactivity of each isolate was confirmed if positive results were reported for the appropriate analyte in 3/3 or 4/5 replicates tested within 10× LoD. If positive results were reported in fewer than 4/5 replicates, additional testing was performed at 100× LoD or higher. Isolates that did not yield the expected results at or below 10× LoD were further investigated to determine the cause of the limitation. Isolates with reactivity limitations will be noted in the SPOTFIRE R/ST Panel product literature.	Analytical reactivity testing demonstrated that the SPOTFIRE R/ST Panel can detect and accurately report results for a diverse collection of isolates from a variety of strains, serotypes, and genotypes of species collected over many years and from geographically distinct locations with few limitations.	The SPOTFIRE R/ST Panel detected and accurately reported results for various species, subspecies, serotypes, and genotypes that may be present in nasopharyngeal and throat swab specimens. The following limitations on reactivity were identified and are noted in the device labeling: • The SPOTFIRE R/ST Panel is intended to detect strains of *Streptococcus dysgalactiae* ssp. *Equisimilis* of human origin. Strains of animal origin lack the region of the genome targeted by the SPOTFIRE R/ST Panel. • Rare strains of *Streptococcus pyogenes* do not contain the region of the genome targeted by the SPOTFIRE R/ST Panel and are not detected.
Analytical Specificity (Exclusivity)	The objective of this study was to assess the analytical specificity (exclusivity) of the SPOTFIRE R/ST Panel by challenging the system with high concentrations of analytes and evaluating the occurrence of unexpected positive results due to assay cross-reactivity. Testing was divided into two categories: on-panel and off-panel. The on-panel exclusivity evaluation assessed the potential for intra-panel cross-reactivity by testing representative organisms that are targeted by the panel. The off-panel exclusivity evaluation assessed the potential for cross-reactivity of panel assays with organisms not detected by the panel.	The primary results evaluated for this study were positive, negative, and uncertain interpretations for each panel analyte. On-panel organisms were expected to have a positive result for the analyte being tested and negative results for all other analytes targeted by the panel. Off-panel organisms were expected to have negative results for all panel analytes, unless otherwise indicated.	Six cross-reactivities were identified by empirical and/or *in silico* evaluations that are predicted to cause inaccurate test results for the SPOTFIRE R/ST Panel. Five of the identified cross-reactivities are either due to reactivity between phylogenetic near-neighbors that are rarely observed in human samples, or reactivity with genomic insertions shared between select species of the same genus. Only one cross-reactivity (HRV/EV assay with select Bordetella species) was due to non-specific interactions between assay primers and sequences within the genome of unrelated organisms.	The SPOTFIRE R/ST Panel assays are specific for the intended analytes, with the following limitations that are noted in the device labeling: • The SARS-CoV-2 assays can amplify select sequences from closely related sarbecoviruses isolated from bats and pangolin. • Some strains of *B. bronchiseptica* contain IS*1001* insertion sequences and will be reported as *Bordetella parapertussis*. • Some *Bordetella* species (*B. pertussis, B. parapertussis,* and *B. bronchiseptica*) will be reported as Human rhinovirus/enterovirus when organisms

Study	Description	Acceptance Criteria	Results	Conclusion
				are present at a high concentration. • The FluA-H1-2009 assay may react with the H1 hemagglutinin gene sequences from influenza A viruses of swine origin. • Some bovine and canine picornaviruses may be reported as Human rhinovirus/ enterovirus. • *Chlamydia gallinacea* may be reported as *Chlamydia pneumoniae.*
Interference (Interfering Substances)	The purpose of this study was to evaluate the potential for relevant substances to interfere with the performance of the assay internal controls or the ability of the system to accurately report test results when analytes were present near the LoD. Substances tested included: • Endogenous substances that may be found in clinical specimens. • Exogenous substances that may be present in clinical specimens, such as medications, treatments, or topical applications. • Technique-specific that could contact specimens during collection or testing. • Microorganisms that could be present as part of normal microbiota or as an infectious organism.	The primary results evaluated for this study were the pass, fail, or invalid results for the internal pouch control assays and analyte positive and negative results. If an unexpected result or control failure was observed for one replicate of a sample containing a potentially interfering substance, the affected sample was retested in two additional pouches to determine if the failure was reproducible.	Accurate results for the SPOTFIRE R/ST Panel were reported in the presence of a variety of potentially interfering substances that may be present in clinical NPS or TS specimens (endogenous substances or microorganisms) or could be introduced during testing (exogenous substances or technique specific substances).	The SPOTFIRE R/ST Panel provides accurate results in the presence of various potentially interfering substances. Although the samples evaluated in this study were not affected by the damaging effects of bleach on nucleic acids, a general warning to avoid contact between samples and bleach is noted in the device labeling.
Near-LoD/ Reproducibility	The purpose of this study was to evaluate the precision (reproducibility) of analyte detection by the SPOTFIRE R/ST Panel and to demonstrate that the SPOTFIRE R/ST Panel could reproducibly provide accurate results for weak-positive and	The primary results evaluated for this study were positive and negative results for each SPOTFIRE R/ST Panel analyte. For all organisms, a minimum of 90% agreement with the expected positive results was required (≥95%	For positive samples, agreement with the expected positive results (all systems/sites) was ≥95% for all analytes. The agreement with the expected negative results	The SPOTFIRE R/ST Panel provides accurate and reproducible analyte detection results over time and in actual use conditions when testing was performed over multiple days, by operators with differing levels of expertise, at

Study	Description	Acceptance Criteria	Results	Conclusion
	negative samples when used by the intended operators at CLIA-waived sites This study was performed on three unique SPOTFIRE Systems at BioFire Diagnostics (BioFire) and at three distinct clinical sites holding a CLIA waiver.	agreement desired) to demonstrate the reproducibility of positive results, and a minimum of 95% agreement with the expected negative results was required.	was 100% for all analytes. The total positive agreement reported for testing completed at BioFire and at clinical sites was nearly identical (99.1% (2052/2070) and 98.9% (1365/1380), respectively) which demonstrates the accuracy and reproducibility of results in the hands of both trained and untrained operators, respectively, and indicates that the use conditions or variables evaluated in the study (site, system, testing day, module, operator, and lot) had no impact on the overall reproducibility of results.	different sites, using different SpotFire Systems and different reagent kit lots. Taken together, the results of this study support use of the SPOTFIRE R/ST Panel and SPOTFIRE System at sites that hold a CLIA Certificate of Waiver.
Matrix Validation	The purpose of this study was to verify the artificial matrices to be used for evaluation of the SPOTFIRE R/ST Panel.	Equivalent performance between the artificial and natural sample matrices was determined primarily based on agreement of positive and negative results at each test concentration. Artificial and natural matrices were considered equivalent if negative results were observed at the same or similar test concentration.	Performance of the SPOTFIRE R/ST Panel was determined to be equivalent in natural and artificial NPS (nNS and aNS) and in natural and artificial throat swab (nTS and aTS) matrices for five representative panel analytes. In all cases, negative results were observed in artificial and natural matrices at the same or similar test concentrations	Equivalent results are achieved when samples are prepared in natural and artificial NPS or natural and artificial TS matrices and tested with the SPOTFIRE R/ST Panel. The results of this study demonstrated that the artificial NPS and artificial TS matrices were acceptable for use in analytical evaluation of SPOTFIRE R/ST Panel performance.
Transport Media Validation	The purpose of this study was to verify that various types of transport media are compatible with the SPOTFIRE R/ST Panel, including BD™ Universal Viral Transport (BD Medical), and Remel MicroTest™ M4RT® Multi-Microbe Media (ThermoFisher).	The primary metric for the determination of compatibility was percent agreement, between the candidate medium and the control medium (CDC VTM formulation) for each spiked analyte at each test concentration. If the overall agreement was 100% when testing above the LoD and ≥95% when testing at the LoD, then the candidate medium was determined to be compatible. It was acceptable for the agreement to be less than 95% when testing below the LoD.	Equivalent analyte detection was observed for all representative analytes when samples were prepared in each of the candidate media types (BD™ Universal Viral Transport, and Remel MicroTest™ M4RT® Multi-Microbe Media) compared to the control medium (CDC VTM).	The SPOTFIRE R/ST Panel demonstrated equivalent results when samples were prepared in Viral Transport Media, BD™ Universal Viral Transport, and Remel MicroTest™ M4RT® Multi-Microbe Media. These transport media are indicated in the product labeling as suitable for use with the SPOTFIRE R/ST Panel.

Study	Description	Acceptance Criteria	Results	Conclusion
Sample Carry Over	The purpose of this study was to evaluate the likelihood of sample-to-sample carry-over during pouch loading and testing of contrived liquid samples.	Positive and negative analyte results were evaluated for each sample. For positive samples, a positive result was expected for the analyte being tested and negative results were expected for all other analytes on the panel. Negative samples were expected to have a negative result for all analytes.	No unexpected positive results were observed in this study.	This study demonstrated that sample-to-sample carry-over between samples containing high concentrations of organism and negative samples is unlikely to occur and that carry-over poses an acceptable risk to the accuracy of the SPOTFIRE R/ST Panel test results when testing is performed according to the instructions for use.

Conclusion:

The fundamental scientific technology, performance, and risk of the SPOTFIRE R/ST Panel are unchanged from the legally marketed SPOTFIRE R Panel. There is no change to the product itself, except for modified software that has been verified and validated to show no change in safety and effectiveness. Therefore, the SPOTFIRE R/ST Panel performs as well as the predicate device.