CLIA Waiver by Application Approval Determination

Decision Memorandum

A. Document Number
CW210006

B. Parent Document Number
K213954

C. CLIA Waiver Type:
Dual 510(k) and CLIA Waiver by Application (Dual Submission)

D. Applicant
BIOFIRE Diagnostics

E. Proprietary and Established Names
BIOFIRE SPOTFIRE Respiratory (R) Panel for use with the BIOFIRE SPOTFIRE System

F. Measurand (analyte)
The BIOFIRE SPOTFIRE Respiratory (R) Panel detects and identifies nucleic acids from the following pathogens: Adenovirus, Seasonal Coronavirus (229E, HKU1, OC43 and NL63 not differentiated), Severe Acute Respiratory Syndrome (SARS)-Coronavirus-2, Human Metapneumovirus, Human Rhinovirus/Enterovirus (not differentiated), Influenza A virus with subtyping of H3 and H1-2009 (reported separately), Influenza B virus, Parainfluenza Virus (serotypes 1-4, not differentiated), Respiratory Syncytial Virus, *Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.

G. Sample Type(s)
Nasopharyngeal swabs

H. Type of Test
Multiplex nucleic acid assay for use with the BIOFIRE SPOTFIRE System for the qualitative detection of viral and/or bacterial pathogens in patients suspected of respiratory tract infection or with signs and/or symptoms of pharyngitis.

I. Test System Description

1. Overview
   The BIOFIRE SPOTFIRE R Panel is a multiplexed nucleic acid-amplification-based test that is intended to detect, identify and differentiate various respiratory viral and bacterial pathogens in nasopharyngeal swab (NPS) specimens from individuals suspected of respiratory tract infection, including COVID-19. The SPOTFIRE R Panel is compatible with the BIOFIRE SPOTFIRE System, an automated polymerase chain reaction (PCR)-based *in vitro* diagnostic system for use with reagent pouches for specific indications.

The BIOFIRE SPOTFIRE System automates nucleic acid extraction and nested multiplex PCR in unitized, closed pouches. The resulting PCR products are evaluated using assay-specific DNA melting analysis. The BIOFIRE SPOTFIRE System Software executes the SPOTFIRE R Panel test and interprets and reports the test results in approximately 15 minutes, without user intervention.

2. Test System Components

The SPOTFIRE System is comprised of between one and four modules that are connected to a single SPOTFIRE Control Station equipped with the SPOTFIRE System Software. The first module is placed on top of the Control Station and additional modules may be stacked on top as required. Each module can be accessed at random to perform a test, independent of the other modules attached to the same Control Station. The SPOTFIRE R Panel Software is required to perform the testing with the SPOTFIRE R Panel.

The BIOFIRE SPOTFIRE R Panel Reagent Kit includes sufficient reagents and consumables to test 30 samples or controls:

- BIOFIRE SPOTFIRE R Panel Pouches (30 ea.)
  - Containing freeze-dried reagents
  - Each pouch is packed under vacuum in a metal canister and outer vacuum-sealed bag
- Sample Preparation Reagent Kit (SPRK) (32 ea.)
  - Individually packaged fixed volume transfer pipette for addition of the test sample to the Sample Injection Vial
  - Sample Buffer ampoule containing ~1 mL of Sample Buffer for addition to the Sample Injection Vial
  - Sample Injection Vial (coded red) for mixing of the test sample and Sample Buffer
  - Hydration Injection Vial (coded blue) containing ~1.5 mL Hydration Solution for pouch rehydration

3. Workflow

Use of the BIOFIRE SPOTFIRE R Panel requires a nasopharyngeal swab (NPS) specimen to be collected according to standard procedures and placed in 1-3 mL of compatible transport medium. The minimum sample volume required to perform a test is 300 µL. Specimens should be tested as soon as possible following collection but may be stored for up to 4 hours at room temperature or for up to 3 days at 2-8 °C or up to 30 days at ≤ -15 °C.

The SPOTFIRE System Software includes step-by-step on-screen instructions that guide the user through the process of starting a run on the instrument.

After cleaning the work area and Pouch Loading Station, the user removes a SPOTFIRE R Panel pouch from its vacuum packaging and places it into the Pouch Loading Station. They then hydrate the pouch using the Hydration Injection Vial by injecting the contents through the Hydration Solution Injection Port, after which they transfer a fixed volume of

sample to the Sample Injection Vial, together with the entire contents of the Sample Buffer ampoule. After mixing the Sample Injection Vial by inversion, the user injects the mixture into the pouch via the Sample Injection Port. The pouch is then inserted in the SPOTFIRE instrument, after which the run starts automatically and proceeds to completion without further user intervention.

Results are interpreted and reported automatically by the system after approximately 15 minutes. The test report is displayed on-screen and may also be printed or saved electronically.

J. Demonstrating "Simple"

- The test uses unitized reagents contained within a sealed pouch. Results are generated automatically following addition of sample and hydration buffer to the reagent pouch and insertion of the pouch into the BIOFIRE SPOTFIRE System.
- The test uses nasopharyngeal swab specimens in a liquid transport medium. An aliquot of the sample is added to the reagent pouch using a fixed volume transfer pipette and Sample Injection Vial that are provided in the kit.
- The test needs only basic, non-technique-dependent specimen manipulation to mix an aliquot of the specimen transport medium with sample buffer and add the mixture to the reagent pouch.
- The test needs only basic, non-technique-dependent reagent manipulation to rehydrate the reagent pouch using the provided Hydration Solution. The sample and reagent injection ports and respective Injection Vials are color coded.
- The test does not require any operator intervention during the analysis steps.
- Technical or specialized training is not required for troubleshooting or error message interpretation. If an error message is shown, on screen instructions are provided to the operator.
- No electronic or mechanical maintenance of the SPOTFIRE instrument is required. The only routine maintenance tasks are periodic cleaning of the exterior surfaces, including the touch screen and to verify that the touch screen angle adjustment paddle is functioning correctly.
- The BIOFIRE SPOTFIRE System analyses and interprets test results automatically. No user calibration is required. The SPOTFIRE System performs self-diagnostics each time power is applied. Malfunctions are reported to the operator as error messages with instructions for appropriate steps.
- The test report is automatically displayed upon completion of a run and can be printed or saved as .PDF file. The report is designed to be easy to understand and includes a Run Summary which includes the sample identity, date, time and operator designation, a Result Summary displaying the results of the test, and a Run Details Summary with additional information including the reagent lot, instrument module and control results for the pouch. Test results are reported as <NEGATIVE>, if none of the panel analytes is detected, or <POSITIVE [Analyte Name(s)]>. In addition, influenza A may be reported as <UNCERTAIN: Influenza A Virus> or <POSITIVE: Influenza A Virus (No Subtype Identified)>, both of which require the sample to be retested once. A result of <INVALID: [Failure reason]> is displayed if there is an instrument or software error,

incomplete or aborted run or internal control failure. An Action Bar with specific instructions to the user is displayed underneath the test results when further action is necessary.

- The SPOTFIRE R Panel is supplied with a Quick Reference Guide (Panel Quick Guide) that was shown to be appropriate for the intended users.

K. Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-safe Mechanisms

1. Risk Analysis

Risk analysis for use of the SPOTFIRE R Panel was performed in accordance with ISO 14971 - *Medical Devices - Application of Risk Management to Medical Devices* by identifying the potential hazards associated with use of the system (false positive, false negative or delayed test results) and the product failure modes that may lead to the identified hazards. The probability of occurrence of a specific failure mode was assigned based on data obtained during the evaluation of clinical performance or real-world evidence. If no data were available, the probability of occurrence was estimated by product experts. For each potential failure mode, the probability of occurrence was assessed before and after risk mitigation. Where possible, design features were modified to reduce or eliminate risks. In addition, appropriate instructions/warnings were added to the product labeling although such measures alone were not considered to be adequate to reduce risk.

After implementation of appropriate controls, all failure modes were mitigated to a low or zero (immeasurable) probability of occurrence (frequency of 1 in 1,000 runs or < 1 in 10,000 runs, respectively). Flex Studies were also performed to evaluate potential variations in testing workflow and to demonstrate the effectiveness of applicable fail-safe or failure alert mechanisms, as described below.

2. Fail-Safe and Failure Alert Mechanisms

The BIOFIRE SPOTFIRE System can be configured with one to four modules, stacked on a control station with an integrated touch screen monitor and barcode reader. The SPOTFIRE System performs self-diagnostic tests including:

1) A Power-on test that is initiated at power-on or when the device or individual modules are reset; and
2) A Run-time test that includes real-time monitoring of operational boundaries and continuously monitors the system components during a run.

If a monitored process is out of specification or otherwise not functioning correctly, an Instrument Error or other status message is displayed.

Table 1 shows the features and functions that are evaluated during the SPOTFIRE System self-tests.

Table 1. BIOFIRE SPOTFIRE System self-monitoring and -diagnostic tests

Process Monitored	Diagnostic Self-Test		Message/Run Status
	Power-on	Run-time	
Inter-board communication	Yes	Yes	Instrument Error/ Invalid
Voltage and current	Yes	Yes	Instrument Error/ Invalid
Pneumatic system compressor and valves, operating pressure	Yes	Yes	Instrument Error/ Invalid
Pouch plunger status (verification of reagent delivery; prevention of pouch re-use)	Yes	Yes	Instrument Error/ Invalid
Bead beater motor	Yes	Yes	Instrument Error/ Invalid
Camera (capture of fluorescence data)	Yes	Yes	Instrument Error/ Invalid
Thermal Control System and heat sink fans	Yes	Yes	Instrument Error/ Invalid
Case fan (modulation of fan speed to maintain operating temperature)	Yes	Yes	Instrument Error/ Invalid
Module Carrier Board (module stack position and cable identification)	Yes	No	Instrument Error/ No Run Initiated
Essential Operating System function	Yes	No	Instrument Error/ No Run Initiated
Pouch Loading Subassembly (loader motor and pouch loading/ejection)	No	Yes	Instrument Error/ Invalid
Internal temperature and humidity	Yes	No	Instrument Error/ No Run Initiated
Operation Environment (prevents operation outside 15-30 °C and > 80% RH)	No	Yes	Operational Environment Out of Range/Invalid

RH: Relative Humidity

The BIOFIRE SPOTFIRE System also includes various fail-safe and failure alert mechanisms that are described in **Table 2** and the effectiveness of which was verified in the Flex Studies described in **Section K(3)**.

Table 2. Fail-safe and failure alert mechanisms

Fail-safe or Failure Alert Mechanism	Description	Failure Modes Prevented
Pouch Controls	• RNA Process Control: freeze-dried *Schizosaccharomyces pombe*; used to monitor lysis, nucleic acid recovery, reverse transcription, PCR stage 1, PCR stage 2 and DNA melt analysis • PCR2 Control: DNA target used to monitor PCR2 and DNA melt analysis • Both controls must produce the expected results for analyte-specific results to be displayed	• Prevent reporting of patient results if any of the monitored processes fail • Prevent generation of patient results if the Hydration and Sample Injection Vials are injected into the wrong ports • Prevent reporting of patient results if inadequate volumes of Hydration Solution and/or Sample Buffer are used • Prevent reporting of patient results if inadequate volume of sample/Sample Buffer is added
Pouch Loading Station Design	• Keyed and color-coded locations for the Hydration and Sample Injection Vials	• Prevents incorrect placement of the Hydration Injection Vial and Sample Injection Vial
Fixed Volume Transfer Pipette	• Used for transfer of sample to the Sample Injection Vial	• Prevents use of excess sample volume
Engineering and Software Controls	• Expired Pouch Lockout	• Prevents initiation of a test with an expired pouch
	• Pouch Re-use Lockout	• Prevents re-testing on the same SPOTFIRE instrument with a previously used reagent pouch
	• Pouch Re-use Instrument Error	• Prevents re-testing on a different instrument of a previously used reagent pouch
	• System Reset	• Prevents a module reset while a test is in progress
	• Operating Environment	• Prevents reporting of patient results if the system operating environment is out of specification (15-30 °C; ≤ 80% relative humidity)

3. Flex Studies

Flex Studies were performed to evaluate the robustness of the BIOFIRE SPOTFIRE System and SPOTFIRE R Panel reagents and to variations in workflow and operating environment that may reasonably be expected to occur with untrained operators in the intended use CLIA Waived setting. Test conditions were designed based on a risk analysis of the complete test system and included conditions intended to verify the effectiveness of in-built controls, lock-out features and failure alerts.

To perform these studies, contrived samples were prepared using artificial nasopharyngeal (aNS) specimen matrix with and without representative on-panel analytes at 3X their respective limit of detection (LoD). Positive samples were tested each day to demonstrate normal operation of the system and thereafter negative samples were tested

under each flex condition to determine whether a valid run could be completed or whether a fail-safe mechanism or failure alert was triggered. If such testing demonstrated activation of an appropriate fail-safe condition or failure alert, the associated engineering controls were determined to be effective and no additional testing was performed. If no engineering controls were triggered, additional testing was performed with positive samples to evaluate the potential for erroneous results. All flex conditions were evaluated using 3 lots of reagents.

The composition of the positive samples used to verify system performance under nominal conditions (controls) and as test materials in subsequent Flex Studies is described in **Table 3**. The analytes were selected to include representative bacteria viruses (DNA, RNA, enveloped, non-enveloped) present on the SPOTFIRE R Panel.

Table 3. Composition of positive samples used in Flex Studies for the BIOFIRE SPOTFIRE System

Analyte	Description	Strain	Source ID	Per mL [1]
Adenovirus B	Non-enveloped DNA virus	Serotype 3	Zeptometrix 0810062CF	2.4 $TCID_{50}$
Human Metapneumovirus	Enveloped RNA virus	B1-3 Peru2-2002	Zeptometrix 0810156CF	0.75 $TCID_{50}$
Parainfluenza Virus 2	Enveloped RNA virus	2	Zeptometrix 08100015CF	42 $TCID_{50}$
Bordetella parapertussis	Gram negative bacterium	E595	Zeptometrix 0801462	120 CFU
Mycoplasma pneumoniae	Intracellular bacterium	M129	Zeptometrix 0810579	30 CCU

[1] 3X Limit of Detection

A brief description of each of the Flex Studies and the associated results is provided in **Table 4**. In most cases, the expected positive or negative results were observed under each of the test conditions, or the in-built fail-safe mechanisms or failure alerts were shown to function as intended to prevent reporting of erroneous results. However, two conditions were identified that were associated with multiple false-negative results for some or all analytes:

Failure to Add Sample to the Sample Injection Vial:
The system was shown to be robust to addition of volumes of sample to the Sample Injection Vial above and below the specified 300 µL (33% to 200%). However, failure to add any sample to the Sample Injection Vial led to false negative results for all analytes. To reduce the likelihood that operators will forget to add sample, the Panel Quick Guide and Instructions For Use instruct operators to add the sample to the Sample Injection Vial prior to addition of Sample Buffer. This workflow was validated in the Clinical Study described below in **Section L** that was conducted at multiple intended use sites, with multiple naïve operators, and in which the clinical performance was shown to be acceptable.

Failure to Add the Specified Volume of Sample/Sample Buffer Mixture to the Pouch: The mixture of Sample and Sample Buffer is drawn into the reagent pouch automatically by vacuum. However, if this process is interrupted prematurely, it is possible to introduce less than the specified volume of the mixture into the reagent pouch, leading to the potential for false negative results. Such results were obtained with a subset of analytes when < ~40% of the specified volume of sample/Sample Buffer was added to the pouch. However, this condition could only be achieved by deliberate misuse of the system and therefore the likelihood of incorrect results due to this failure mode is considered low. As above, the SPOTFIRE R Panel workflow was validated in the Clinical Study with naïve users and performance was shown to be acceptable.

Overall, the Flex Studies demonstrated that the SPOTFIRE R Panel is robust to foreseeable user-dependent variations in the assay workflow and that in-built assay controls and fail-safe and/or failure alert mechanisms are effective in preventing the generation of erroneous results due to operator error and/or use of the BIOFIRE SPOTFIRE System outside the specified operating environmental conditions.

Table 4. Summary of Flex Studies and fail Safe/failure alert verification testing

Specification	Test Condition	Fail Safe/ Failure Alert	Agreement		Potential for Erroneous Results
			Negative	Positive	
Reagent shelf-life	Attempt to use expired reagent kit/pouches	Yes	Lockout activated	N/A	No
Reagent storage temperature (15-25 °C)	Storage of reagents for ~24 hours outside the specified temperature range				
	-20 °C	No	3/3	6/6	No
	2-8 °C	No	3/3	11/12 [1]	No
	≥ 40 °C	No	3/3	6/6	No
Reagent pouch should be loaded within 30 minutes of removal from vacuum packaging	Removal of reagent pouch from packaging and hold for ~8 hours at 15-25 °C	No	3/3	6/6	No
Pause 5 seconds after unscrewing the Sample Injection Vial to prevent dripping	Alternative positive and negative samples tested without the specified 5 second delay or between-sample cleaning	No	6/6	6/6	No
Instrument run should be initiated within ~60 minutes of loading the pouch	Pouch held for ~ 8 hours after hydration and sample loading				
	15 - 25 °C	No	3/3	6/6	No
	15 - 25 °C under UV light	Yes	3/3 Invalid	N/A	No
	-20 °C	No	3/3	6/6	No
	2-8 °C	No	3/3	6/6	No
	40 °C	No	3/3	6/6	No
Reagent pouch is robust to normal handling	Pouch dropped after loading	No	3/3	6/6	No
Hydration Injection Vial and Sample Injection Vial are keyed to prevent misuse	Incorrect placement of vials on the Pouch Loading Station	Yes	Form factor and color coding prevent incorrect placement		No
Addition of sample to the Sample Injection Vial using the supplied transfer pipette	Addition of an incorrect volume of sample to the Sample Injection Vial				

Specification	Test Condition	Fail Safe/ Failure Alert	Agreement		Potential for Erroneous Results
			Negative	Positive	
	No sample	No	3/3	**0/6**	**Yes** [2]
	100 µL ((33%)	No	3/3	6/6	No
	600 µL (200%)	No	3/3	6/6	No
Addition of ~800 µL of Sample Buffer to the Sample Injection Vial	Failure to add specified volume of Sample Buffer				
	No Sample Buffer	Yes	3/3 Invalid	N/A	No
	100 µL (12.5%)	No	3/3	6/6	No
Operator inverts the Sample Injection Vial 3X to mix	Failure to mix or excessive mixing				
	No mixing	No	3/3	6/6	No
	5X inversion	No	3/3	6/6	No
	Vigorous shaking	No	3/3	6/6	No
Application of the Hydration and Sample Injection Vials to the correct ports	Use of the Hydration Injection Vial in the Sample Port and the Sample Injection Vial in the Hydration Port	Yes	3/3 Invalid	N/A	No
The specified volume of Hydration Solution is drawn into the pouch automatically by vacuum	Failure to rehydrate the pouch with the correct volume of hydration solution				
	No Hydration Solution added	Yes	3/3 Invalid	N/A	No
	20-45 % of target hydration volume added by weight	Yes	3/3 Invalid	N/A	No
The specified volume of Sample/Sample Buffer mixture (~300 µL) is drawn into the pouch by vacuum	Failure to add correct volume of Sample/Sample Buffer mixture to the pouch				
	No Sample/Sample Buffer	Yes	3/3 Invalid	N/A	No
	12.5 - 60% target volume by weight	No	3/3	**4/6** [3]	**Yes** [2]
Reagent pouch is single use	Attempt to re-use a spent pouch				
	On the same SPOTFIRE System	Yes	Lockout Activated	N/A	No
	On a different SPOTFIRE System	Yes	Instrument Error	N/A	No
Ability to reset System/module to clear errors	Inadvertent or deliberate misuse of the reset button	Yes [3]	3/3	6/6	No
Ability to configure the SPOTFIRE System for 1 to 4 modules	Removal and re-addition of a module during a run				
	Inactive module	No	3/3	6/6	No
	Active module	No	3/3	6/6	No
Operation of the SPOTFIRE System in a fixed, upright position on a level surface	Movement of the SPOTFIRE System during operation or operation on a non-level surface				
	Sliding of the system (12" in ≤ 20 sec)	No	3/3	6/6	No
	Operation at a 20 ° angle from vertical	No	3/3	11/12 [4]	No
The SPOTFIRE System is intended to operate between 15 and 30 °C and 15 to 80 % RH	Operation out of the specified ranges of temperature/RH				
	Low/Ambient 9.9-11.4 °C; 42.9-50.3 % RH	Yes	Temperature Out of Range		No
	Low/Low 9.0-10.0 °C; 15.3-16.5 % RH	Yes	Temperature Out of Range		No
	High/Ambient 34.9-35.0 °C; 47.9-49.1 % RH	Yes	Temperature Out of Range		No
	High/Low 34.7-34.9 °C; 8.5-9.9 % RH	Yes	Temperature Out of Range		No

Specification	Test Condition	Fail Safe/ Failure Alert	Agreement		Potential for Erroneous Results
			Negative	Positive	
	Low/High 10.1-10.8 °C; 79.6-86.6 % RH	Yes	Temperature and/or Humidity Out of Range		No
	Ambient/High 21.1-25.3 °C; 85.5-88.5 % RH	Yes	Humidity Out of Range		No
	High/High 34.6-34.8 °C; 83.4-84.4 % RH	Yes	Temperature and/or Humidity Out of Range		No
	Ambient/Low 14.6-19.5 °C; 0.4-6.0 % RH	No	3/3	6/6	No
The SPOTFIRE System is intended to operate at an elevation ≤ 10,000 feet above sea level	Operation at 10,400 feet above sea level	No	3/3	6/6	No
The SPOTFIRE System is robust to vibration	System operated near equipment generating high or low frequency vibration				
	Low frequency (1.04-2.00 mm/s; 0.043-0.079 g)	No	3/3	6/6	No
	High frequency (0.92-1.09 mm/s; 0.089 g)	No	3/3	6/6	No

N/A: Not Applicable; RH: Relative Humidity
Invalid: Control Failure

[1] 1/6 replicates reported negative for Human Metapneumovirus and Parainfluenza Virus 2 on initial testing; 6 additional replicates all produced the expected results and therefore the likelihood of an incorrect result is considered low

[2] The workflow for the SPOTFIRE R Panel was validated in a prospective Clinical Study with naïve operators and shown to be robust to user error. Therefore, in practice, the likelihood of obtaining false results due to this failure mode is considered low.

[3] 2/6 replicates reported negative for Human Metapneumovirus and Parainfluenza Virus 2

[3] Reset not permitted while a run is in progress

[4] 1/6 replicates negative for Human Metapneumovirus on initial testing; 6 additional replicates all produced the expected results and therefore the likelihood of an incorrect result is considered low

Specimen Stability

The stability of the BIOFIRE R/ST Panel analytes in nasopharyngeal swab matrix in Viral Transport Medium (VTM) was established through analytical studies. The claimed stability of such specimens for use with the SPOTFIRE R Panel is 4 hours at 15-25 °C, 3 days at 2-8 °C and 30 days at < -15 °C. To support use of the SPOTFIRE R Panel in a CLIA Waived setting, additional testing was performed to demonstrate the robustness of the assay system to storage of specimens under conditions outside those recommended in the device labeling, as described below.

Natural nasopharyngeal swab matrix from asymptomatic volunteer subjects was spiked with representative bacterial and viral analytes included on the SPOTFIRE R Panel (**Table 5)**. All samples were confirmed to be negative for the target analytes prior to use in preparation of contrived specimens for the study. Ten replicates of each sample were tested immediately after preparation and a further 10 replicates were tested after storage at 30 °C for 4 hours, 25 °C for 8 hours or 8 °C for 7 days. All samples produced the expected positive results for each of the target analytes (10/10 positive results, 100%). The SPOTFIRE R Panel appears robust to the conditions to which specimens are likely to be exposed prior to testing in the intended use environment.

Table 5. Panel members used in evaluation of robustness to the conditions of specimen storage

Sample	Species/Strain	Source Identity	Concentration per mL [1]
1	Adenovirus B Serotype 3	Zeptometrix 0810062CF	2.4 $TCID_{50}$
	Coronavirus NL63	Zeptometrix 0810228CF	0.0075 $TCID_{50}$
	Human Enterovirus D68 US/MO/14-18947	ATCC VR-1823	33 $TCID_{50}$
	Influenza A H3N2 Hong Kong/4801/14	Zeptometrix 0810526CF	2.6 $TCID_{50}$
	Influenza B Florida/02/06	Zeptometrix 0810037CF	0.099 $TCID_{50}$
	Bordetella pertussis A639	Zeptometrix 0801459	990 CFU
	Mycoplasma pneumoniae M129	Zeptometrix 0801579	30 CCU
2	Influenza A H1N1pdm Michigan/45/15	Zeptometrix 0810538CF	2.5 $TCID_{50}$
	Human metapneumovirus 18 Type B2 IA18-2003	Zeptometrix 0810162CF	6.0 $TCID_{50}$
	Parainfluenza Virus Type 2	Zeptometrix 0810015CF	42 $TCID_{50}$
	Respiratory Syncytial Virus A	Zeptometrix 0810040ACF	0.19 $TCID_{50}$
	Bordetella parapertussis E595	Zeptometrix 0801462	120 CFU
	Chlamydia pneumoniae AR-39	ATCC 53592	60 IFU

CCU: Color changing Units; CFU: Colony Forming Units; IFU: Inclusion Forming Uunits; $TCID_{50}$: Tissue Culture Infectious Dose-50%

[1] 3X LoD

L. Demonstrating "Insignificant Risk of an Erroneous Result" –Accuracy

1. Comparision Study

a. Study Design

i. Study Sites and Duration

The performance of the SPOTFIRE R Panel in the hands of untrained users was evaluated in a Prospective Clinical Study that was performed at five sites, four of which were in the U.S. and one of which was in the U.K. All five sites followed the same protocols and procedures, were subject to the same monitoring process, and were considered representative of CLIA Waived intended use sites. The sites included three adult emergency departments and two pediatric urgent care and/or emergency departments. Specimen enrollment began in December, 2020 and

concluded in September, 2021. Specimens were either collected under informed consent or were residual specimens that were left over from standard of care testing.

The Prospective Clinical Study was supported by additional testing at the four U.S. sites that was performed on frozen, archived specimens obtained from various clinical laboratories distributed worldwide. These specimens were selected for inclusion in the study based on their known microbial content as determined by the source laboratory, and which was confirmed using the same comparator methods as for the prospectively collected specimens.

ii. Operators

Prospective Clinical Study
A total of 29 operators participated in the Prospective Clinical Study, with between 1 an 15 operators per site (**Table 6**). The participating operators were selected from a pool of available non-laboratory personnel with diverse educational and work experience who were considered representative of untrained, naïve operators in the intended use setting. No hands-on training was given to the SPOTFIRE R Panel test operators who were only provided with the Panel Quick Guide. A single operator at each site was selected at random to perform instrument installation using the System Setup Quick Guide.

Of the 29 operators who participated in the Prospective Clinical Study, 24 (82.8%) processed at least 5 nasopharyngeal samples that were positive for one or more of the targeted respiratory analytes, as determined by the applicable comparator methods.

Table 6. Summary of samples tested in the Prospective Clinical Study, stratified by site and operator

Site	Operator	Nasopharyngeal Swabs		
		Tested	Positive [1]	%
1	01	361	110	30.5
	Sub-total	**361**	**110**	**30.5**
2	01	19	8	42.1
	03	10	5	50.0
	05	7	4	57.1
	Sub-total	**36**	**17**	**47.2**
3	01	17	13	76.5
	02	47	37	78.7
	04	16	11	68.8
	05	62	43	69.4
	06	16	9	56.3
	07	28	18	64.3
	08	56	42	75.0
	09	6	4	66.7
	10	44	33	75.0
	11	57	40	70.2
	12	36	21	58.3
	13	58	40	69.0
	14	8	8	100
	15	47	22	46.8
	16	13	9	69.2
	Sub-total	**511**	**350**	**68.5**
4	01	32	18	56.3
	03	14	13	92.9
	04	41	26	63.4
	05	38	33	86.8
	06	22	18	81.8
	Sub-total	**147**	**108**	**73.5**
5 [2]	01	4	1	25.0
	02	10	1	10.0
	03	33	18	54.5
	04	16	6	37.5
	06	2	0	0.0
	Sub-total	**65**	**26**	**40.0**
	Total	**1120**	**611**	**54.6**

[1] Positive for at least one target analyte by the applicable comparator method

[2] Ex-U.S. site

A post instrument set-up questionnaire and a post-study questionnaire were administered to the participating operators. Of the 12 individuals who took part in SPOTFIRE System installation (which included Quality Control testing), all 12 (100%) reported that the instrument assembly was either “Easy” or “Very Easy”. Overall, the study participants reported that testing with the SPOTFIRE R Panel was easy to perform using the System Setup and Panel Quick Guides, without the need for additional training materials.

Archived Specimen Testing

Testing of Archived Specimens with known microbial content was performed by 17 of the operators who participated in the Prospective Clinical Study, representing each of the four U.S. study sites. Included were 542 nasopharyngeal swabs of which the majority were positive for at least one analyte. All but two participants processed more than 5 positive samples (**Table 7**).

Table 7. Summary of testing performed with Archived Specimens, stratified by site and operator

Site	Operator	Nasopharyngeal Swabs		
		Tested	Positive [1]	%
1	01	97	86	88.7
	Sub-total	**97**	**86**	**88.7**
2	01	15	15	100
	03	20	18	90.0
	04	1	1	100
	05	44	40	90.9
	Sub-total	**80**	**74**	**92.5**
3	02	31	30	96.8
	05	5	4	80.0
	07	42	40	95.2
	11	20	19	95.0
	12	24	21	87.5
	13	50	48	96.0
	15	38	35	92.1
	Sub-total	**210**	**197**	**93.8**
4	01	20	19	95.0
	03	12	11	91.7
	04	36	33	91.7
	05	14	12	85.7
	06	73	68	93.2
	Sub-total	**155**	**143**	**92.3**
	Total	**542**	**500**	**92.3**

N/A: Not applicable

[1] Positive for at least one target analyte by the applicable comparator method

iii. Instructions for Use

Operators who participated in evaluating the clinical performance of the SPOTFIRE R Panel did not receive any training on how to perform the assay and were instructed to refer solely to the SPOTFIRE R Panel Quick Guide. Telephone technical support was provided as intended for the commercial product.

iv. Subjects (Patients)

The Prospective Clinical Study included specimens that were collected under consent as well as residual specimens leftover from standard of care testing, as decribed below.

Specimen Collection Under Consent
Specimens for the Prospective Clinical Study were collected under informed consent (if required by the local Institutional Review Board) or, if the subject was < 18 years of age, with parental permission and assent according to the following Inclusion Criteria:

Subjects who:

- Presented with signs/symptoms of a respiratory infection including but not limited to fever, cough, sore throat, runny nose, myalgia, headache, chills, or fatigue.
- Were willing and able to provide a nasopharyngeal swab specimen.

The Exclusion Criteria for the study were as follows:

- Inability or unwillingness to provide informed consent (if required) or parental permission and assent.
- Inability or unwillingness to provide the required specimen.
- The subject's healthcare provider determined that specimen collection represented an unacceptable health risk.

Residual Specimens
Residual specimens were exempt from Informed Consent requirements but were required to meet the following Inclusion Criteria:

- Residual nasopharyngeal swab specimen leftover from standard of care testing under a clinician order for analysis for respiratory pathogens.
- Specimen held ≤ 4 hours at room temperature or ≤ 72 hours at 4 °C.
- Residual volume available ≥ 1.5 mL.

The Exclusion Criteria for residual specimens were as follows:

- Specimen could not be tested within the defined parameters of storage.
- Insufficient volume available for testing.
- Type of transport medium unknown.

v. Samples

The clinical performance of the SPOTFIRE R Panel was evaluated using a combination of prospectively collected and archived specimens as described below.

Prospectively Collected Specimens

A Clinical Study with prospectively collected specimens was conducted at five sites (4 U.S. and 1 ex-U.S.), including adult and pediatric emergency departments/urgent care centers, that were considered representative of the intended use settings for the BIOFIRE SPOTFIRE System and SPOTFIRE R Panel. The specimens included in the study were either collected under Informed Consent (if required by the participating institution), or with parental permission for minors < 18 years of age, or were leftover (residual) samples from standard of care testing. The inclusion and exclusion criteria for the study are summarized in **Table 8**. Aliquots of each specimen were prepared for testing with the BIOFIRE SPOTFIRE R Panel and the applicable comparator method(s).

Table 8. Inclusion and exclusion criteria for the Prospective Clinical Study

Prospectively Collected Specimens	
Inclusion Criteria	Subject presented with signs/symptoms of respiratory infection included but not limited to fever, cough, sore throat, runny nose, myalgia, headache, chills or fatigue
	If ≥ 18 years of age subject provided Informed Consent [1] If < 18 years of age, parental permission and assent obtained [1]
	Subject willing to provide 1 nasopharyngeal swab specimen
Exclusion Criteria	Subject is unable or unwilling to provide Informed Consent or parental assent (if required)
	Subject is unable or unwilling to provide the required specimens
	Subject's healthcare provider determined that specimen collection represented an unacceptable healthcare risk
Residual Specimens [2]	
Inclusion Criteria	Residual nasopharyngeal swab specimen leftover from standard of care testing under a physician order for analysis for respiratory pathogens
	Specimen held at room temperature for < 4 hours or at 4 °C for ≤ 72 hours
	≥ 1.5 mL specimen volume available
Exclusion Criteria	Specimen could not be tested within the specified storage parameters
	Insufficient specimen volume for testing
	Type of transport medium not known

[1] The Institutional Review Board at one site waived the need for a signed Informed Consent form

[2] Residual specimens were exempt from Informed Consent requirements in accordance with FDA's guidance document "Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable" (April, 2006)

A total of 1215 nasopharyngeal swab specimens were initially enrolled in the Prospective Clinical Study, of which 95 were excluded from the analysis of performance for the reasons listed in **Table 9**. In addition, some specimens were excluded from the analysis of performance for one or more specific analytes due to improper storage of sample aliquots or failure or inappropriate conduct of the comparator method and inability to retest due volume constraints. All nasopharyngeal swabs included in the analysis of performance were collected in viral transport medium (VTM).

Table 9. Summary of data exclusions from the Prospective Clinical Study

Rationale for Exclusion	Number of Specimens (n = 1215)
Specimen did not meet inclusion criteria	4
Inappropriate volume of transport medium	0
SPOTFIRE R Panel could not be performed within the specified time	1
Specimen handling error	4
Specimen not aliquoted within the specified time	8
Invalid SPOTFIRE R Panel result	38
Specimen not received for comparator testing	29
Use of expired SPOTFIRE R PANEL reagents [1]	11
Total Excluded	**95**
Total Included	**1120**

[1] Reagents labeled with incorrect expiration date, preventing application of lock-out feature

Archived Specimens

Several analytes included on the SPOTFIRE R Panel were not encountered during the Prospective Clinical Study in sufficient numbers to demonstrate system performance. Therefore, the Prospective Clinical Study was supported by additional testing at the four U.S. study sites that was performed on frozen, archived specimens obtained from various clinical laboratories from around the world. These specimens were selected for inclusion in the study based on their microbial content as originally determined by the source laboratory and were stored frozen prior to testing. The microbial content of the archived specimens was confirmed using the same molecular comparator methods as for the prospectively collected specimens. A minimum sample volume of 650 µL was required for confirmatory testing to be performedPanels.

A total of 562 archived nasopharyngeal swab specimens, of which, 20 were excluded from the analysis of performance due to invalid SPOTFIRE R Panel results or to invalid or missing comparator test results (**Table 10**).

Table 10. Summary of data exclusions from the testing archived clinical specimens

Rationale for Exclusion	Number of Specimens
Invalid SPOTFIRE R Panel Result	12
Invalid or missing comparator test result	8
Total	**20**

vi. Comparative Method (CM)

A description of each of the comparator methods used to establish the performance of the SPOTFIRE R Panel is provided in **Table 11**.

Table 11. Comparator methods used with prospectively collected and archived clinical specimens

SPOTFIRE R Panel Assay	FDA-Cleared Comparator Method
Adenovirus	Multi-Analyte Panel-1 [3]
Coronavirus Seasonal	
Human Metapneumovirus	
Human Rhinovirus/Enterovirus	
Influenza A Virus	
Influenza A Virus A/H1-2009	
Influenza A Virus A/H3	
Parainfluenza Virus	
Respiratory Syncytial Virus	
Bordetella pertussis	
Chlamydia pneumoniae	
Mycoplasma pneumoniae	
Coronavirus SARS-CoV-2 [1]	Multi-Analyte Panel-2
Influenza B Virus [2]	
Bordetella parapertussis [1]	

[1] Not detected by Multi-Analyte Panel-1 comparator

[2] Due to reduced sensitivity of the Multi-Analyte Panel-1 for influenza B, Multi-Analyte Panel-2 was used as the comparator for this analyte

[3] Separate results reported for coronaviruses 229E, HKU1, NL63 and OC43, as well as parainfluenza virus serotypes 1 - 4

b. Results and Analysis

i. Statistical Analysis of Comparison Study Results

A summary of the results from testing prospectively collected and archived nasopharyngeal swabs is shown in **Table 12**. Positive Percent Agreement (PPA) ranged from 96.3-100% for prospectively collected specimens and from 96.0-100% for archived specimens, depending on the analyte, whereas Negative Percent Agreement (NPA) ranged from 90.6-100% for prospectively collected specimens and from 96.7-100% for archived specimens. The only analyte for which NPA was < 95% was Human Rhinovirus/Enterovirus with prospectively collected specimens. However, additional testing provided evidence for the presence of Human Rhinovirus/Enterovirus in 48/72 specimens (66.7%) with discordant positive SPOTFIRE R Panel results and therefore the low NPA with this analyte was considered acceptable.

Overall, the SPOTFIRE R Panel exhibited acceptable PPA and NPA in comparison to other FDA-cleared methods for the detection of the targeted analytes in nasopharyngeal swab specimens.

Table 12. Performance of the SPOTFIRE R Panel with prospectively collected and archived clinical specimens

Viruses					
Assay	**Study**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
Adenovirus	Prospective	97.0 (84.7-99.5)	32/33	97.8 (96.7-98.5)	1058/1082
	Archived	100 (89.0-100)	31/31	96.9 (94.9-98.2)	439/453
Coronavirus Seasonal	Prospective	99.0 (94.7-99.8)	101/102	98.7 (97.8-99.2)	1000/1013
	Archived	99.0 (94.3-99.8)	95/96	98.2 (96.3-99.1)	381/388
Coronavirus SARS-CoV-2	Prospective	97.3 (90.5-99.2)	71/73	99.4 (98.7-99.7)	1031/1037
	Archived	N/A	N/A	N/A	N/A
Human Metapneumovirus	Prospective	100 (20.7-100)	1/1	100 (99.7-100)	1114/1114
	Archived	97.0 (84.7-99.5)	32/33	100 (99.2-100)	451/451
Human Rhinovirus/Enterovirus	Prospective	99.1 (97.5-99.7)	345/348	90.6 [1] (88.3-92.5)	695/767
	Archived	96.7 (83.3-99.4)	29/30	96.7 (94.6-98.0)	439/454
Influenza A virus	Prospective	N/A	N/A	100 (99.7-100)	1115/1115
	Archived	98.3 (91.0-99.7)	58/59	100 (99.1-100)	423/423
Influenza A Virus A/H1-2009	Prospective	N/A	N/A	100 (99.7-100)	1115/1115
	Archived	96.9 (84.3-99.4)	31/32	100 (99.2-100)	450/450
Influenza A Virus A/H3	Prospective	N/A	N/A	100 (99.7-100)	1115/1115
	Archived	100 (87.5-100)	27/27	100 (99.2-100)	455/455
Influenza B Virus	Prospective	N/A	N/A	100 (99.7-100)	1110/1110
	Archived	100 (88.7-100)	30/30	100 (87.9-100)	28/28
Parainfluenza Virus	Prospective	98.0 (92.9-99.4)	96/98	98.9 (98.1-99.4)	1006/1017
	Archived	98.3 (94.0-99.5)	116/118	98.1 (96.1-99.1)	359/366
Respiratory Syncytial Virus	Prospective	96.3 (81.7-99.3)	26/27	99.8 (99.3-99.9)	1086/1088

	Archived	100 (90.6-100)	37/37	98.4 (96.8-99.2)	440/447
Bacteria					
Analyte	**Study**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
Bordetella parapertussis	Prospective	N/A	N/A	100 (99.7-100)	1110/1110
	Archived	96.0 (80.5-99.3)	24/25	100 (89.6-100)	33/33
Bordetella pertussis	Prospective	N/A	N/A	100 (99.7-100)	1115/1115
	Archived	96.4 (82.3-99.4)	27/28	99.1 (97.8-99.7)	452/456
Chlamydia pneumoniae	Prospective	N/A	N/A	100 (99.7-100)	1115/1115
	Archived	100 (88.7-100)	30/30	99.6 (98.4-99.9)	452/454
Mycoplasma pneumoniae	Prospective	N/A	N/A	100 (99.7-100)	1115/1115
	Archived	100 (89.6-100)	33/33	98.9 (97.4-99.5)	446/451

PPA: Positive Percent Agreement; NPA: Negative Percent Agreement: 95% CI: 95% score confidence interval; TP: True Positive; FP: False Positive; False Negative; True Negative (all as determined with respect to the comparator); N/A: Not Applicable

[1] 48/72 specimens (66.7%) were determined to be positive for Human Rhinovirus/Enterovirus upon retesting with the same FDA-cleared comparator (1), by testing with either an alternative FDA-cleared assay (42) or with PCR followed by bidirectional sequencing (5)

ii. Device Performance with Analyte Concentrations Near the Cutoff

The reproducibility and precision of the SPOTFIRE R Panel on different BIOFIRE SPOTFIRE Systems was evaluated with multiple operators who tested positive and negative samples over multiple days at three study sites, in addition to BIOFIRE Diagnostics. All analytes detected by the panel were evaluated using contrived positive specimens at concentrations equivalent to ≤ 3X their respective analytical limit of detection (LoD) (**Table 13**). Negative samples were prepared using artificial matrix only.

Table 13. Contrived samples used to evaluate the precision/reproducibility of the SPOTFIRE R Panel

Sample	Analyte/Strain	Source Identity	Concentration (per mL)
1	None	--	--
2	*Bordetella pertussis* A639	Zeptometrix 0801459	990 CFU
	Mycoplasma pneumoniae M129	Zeptometrix 0801579	10 CCU
	Adenovirus Species B Type 3	Zeptometrix 0810062CF	2.4 $TCID_{50}$
	Coronavirus NL63	Zeptometrix 0810228CF	0.0025 $TCID_{50}$
	Influenza B B/Florida/02/06	Zeptometrix 0810037CF	0.099 $TCID_{50}$
	Parainfluenza virus 4a	Zeptometrix 0810060CF	200 $TCID_{50}$
3	*Bordetella parapertussis* E595	Zeptometrix 0801462	120 CFU
	Chlamydophila pneumoniae AR-39	ATCC 53592	20 IFU
	Human metapneumovirus 3 Type B1	Zeptometrix 0810156CF	0.75 $TCID_{50}$
	Parainfluenza virus 1	Zeptometrix 0810014CF	4.6 $TCID_{50}$
	Influenza A H1N1pdm Michigan/45/15	Zeptometrix 0810538CF	2.5 $TCID_{50}$
	Coronavirus SARS-CoV-2 2019-nCoV/USA-WA1/2020	ATCC VR-1986HK	250 copies
4	Human enterovirus D68 US/MO/14-18947	ATCC VR-1823	11 $TCID_{50}$
	Parainfluenza virus 2	Zeptometrix 0810015CF	42 $TCID_{50}$
	Respiratory syncytial virus A 2006	Zeptometrix 0810040ACF	6.2 x 10^{-2} $TCID_{50}$
	Coronavirus 229E	ATCC VR-740	2.0 $TCID_{50}$
5	Parainfluenza virus 3	Zeptometrix 0810016CF	8.8 $TCID_{50}$
	Influenza A H3N2 Hong Kong/4801/14	Zeptometrix 0810526CF	2.6 $TCID_{50}$
	Coronavirus OC43	Zeptometrix 0810024CF	1.6 x 10^{-2} $TCID_{50}$

CCU: Color Changing Units; $CEID_{50:}$ Chicken Egg Infectious Dose-50%; CFU: Colony Forming Units; IFU: Inclusion Forming Units; $TCID_{50}$: Tissue Culture Infectious Dose-50%

At BIOFIRE Diagnostics, testing was performed on three SPOTFIRE Systems, using three lots of reagents over five consecutive days. On each day of testing, two operators each tested three replicates of each sample (one per reagent lot) on each SPOTFIRE System for a total of 90 replicates per sample overall (2 operators x 3 samples x 3 instruments x 5 days = 90 replicates).

At the external study sites, testing was performed with a single SPOTFIRE System at each site and occurred over five non-consecutive days using a single lot of reagents that was common across each of the sites. On each day, two untrained

operators each tested two replicates of each sample for a total of 60 replicates per sample overall (2 operators x 2 samples x 3 sites x 5 days = 60 replicates).

Precision and reproducibility for each analyte were calculated by assessing the agreement between the reported test results and the expected results for each sample.

A summary of the results of the study, stratified by analyte and SPOTFIRE system/site is provided in **Table 14**. Positive agreement for all analytes was > 95% and there was negligible difference between instruments, sites, reagent lots or operators. Negative agreement was 100% in all cases.

Overall, the reproducibility and precision of the SPOTFIRE R Panel for detection of the target analytes was determined to be acceptable.

Table 14. Summary of results from the Precision and Reproducibility Study, stratified by SPOTFIRE System and study site

Assay/Analyte		Expected Result	Agreement (%) Stratified by Site and SPOTFIRE System						Total
			BIOFIRE Diagnostics			External Site			
			A	B	C	1	2	3	
Adenovirus		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
Adenovirus Species B Type 3		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Bordetella parapertussis		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
B. parapertussis E595		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Bordetella pertussis		Positive	29/30 (96.7)	29/30 (96.7)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	148/150 (98.7)
			88/90 (97.8)			60/60 (100)			
B. pertussis A639		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Chlamydia pneumoniae		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
Chlamydophila pneumoniae AR-39		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Seasonal Coronavirus	Coronovirus 229E	Positive	30/30 (100)	30/30 (100)	29/30 (96.7)	20/20 (100)	20/20 (100)	20/20 (100)	149/150 (99.3)
			89/90 (98.9)			60/60 (100)			
	Coronavirus OC43	Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
	Coronavirus NL63	Positive	27/30 (90.0)	30/30 (100)	30/30 (100)	18/20 (90.0)	20/20 (100)	18/20 (90.0)	143/150 (95.3)
			87/90 (96.7)			56/60 (93.3)			

Assay/Analyte		Expected Result	Agreement (%) Stratified by Site and SPOTFIRE System						
			BIOFIRE Diagnostics			External Site			Total
			A	B	C	1	2	3	
	None	Negative	60/60 (100)	60/60 (100)	60/60 (100)	40/40 (100)	40/40 (100)	40/40 (100)	300/300 (100)
			180/180 (100)			120/120 (100)			
Coronavirus SARS-CoV-2 SARS-CoV-2 2019-nCoV/USA-WA1/2020		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Human Metapneumovirus hMPV 3 Type B1		Positive	30/30 (100)	30/30 (100)	30/30 (100)	19/20 (95.0)	20/20 (100)	19/20 (95.0)	148/150 (98.7)
			90/90 (100)			58/60 (96.7)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Human Rhinovirus/ Enterovirus Enterovirus D68 US/MO/14-18947		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Influenza A Virus	Influenza A H1N1pdm	Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
	Influenza A H3N2	Positive	30/30 (100)	30/30 (100)	30/30 (100)	19/20 [3] (95.0)	19/20 (95.0)	20/20 (100)	148/150 (98.7)
			90/90 (100)			58/60 (96.7)			
	None	Negative	90/90 (100)	90/90 (100)	90/90 (100)	60/60 (100)	60/60 (100)	60/60 (100)	450/450 (100)
			270/270 (100)			180/180 (100)			
Influenza A Virus Subtype H1-2009 [1] Influenza A H1N1pdm Michigan/45/15		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Influenza A Virus Subtype H3 [2] Influenza A H3N2 Hong Kong/4801/14		Positive	30/30 (100)	30/30 (100)	30/30 (100)	19/20 [3] (95.0)	19/20 (95.0)	20/20 (100)	148/150 (98.7)
			90/90 (100)			58/60 (96.7)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Influenza B Virus Influenza B B/Florida/02/06		Positive	29/30 (96.7)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	149/150 (99.3)
			89/90 (98.9)			60/60 (100)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Mycoplasma pneumoniae *M. pneumoniae* M129		Positive	29/30 (96.7)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	149/150 (99.3)
			89/90 (98.9)			60/60 (100)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Parainfluenza Virus	Parainfluenza Virus 1	Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)

Assay/Analyte		Expected Result	Agreement (%) Stratified by Site and SPOTFIRE System						
			BIOFIRE Diagnostics			External Site			Total
			A	B	C	1	2	3	
			90/90 (100)			60/60 (100)			
	Parainfluenza Virus 2	Positive	30/30 (100)	29/30 (96.7)	28/30 (93.3)	20/20 (100)	20/20 (100)	20/20 (100)	147/150 (98.0)
			87/90 (96.7)			60/60 (100)			
	Parainfluenza Virus 3	Positive	28/30 (93.3)	30/30 (100)	30/30 (100)	19/20 (95.5)	18/20 (90.0)	20/20 (100)	145/150 (96.7)
			88/90 (97.8)			57/60 (95.0)			
	Parainfluenza Virus 4	Positive	29/30 (96.7)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	149/150 (99.3)
			89/90 (98.9)			60/60 (100)			
	None	Negative	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
Respiratory Syncytial Virus RSV A 2006		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	19/20 (95.0)	20/20 (100)	149/150 (99.3)
			90/90 (100)			59/60 (98.3)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Positive Agreement By System/Site			**621/630 (98.6)**	**628/630 (99.7)**	**627/630 (99.5)**	**414/420 (98.6)**	**415/420 (98.8)**	**417/420 (99.3)**	**3122/ 3150 (99.1)**
			1876/1890 (99.3)			**1246/1260 (98.9)**			

[1] 1 positive sample was initially reported as Positive: Influenza A Virus (No Subtype Found) and was therefore re-tested. The re-test result was as expected. The retest results from this sample for each analyte are included in the summary table.

[2] 2 positive samples were initially reported as Positive: Influenza A Virus (No Subtype Found) and 1 positive sample was initially reported as Uncertain: Influenza Virus. All 3 samples were retested and the retest result were as expected. The retest results from these samples for each analyte are included in the summary table.

[3] 1 sample was reported as Uncertain Influenza Virus but was not retested.

2. Operator Questionnaire

Prior to the start of the Clinical Study, all participating personnel were asked to complete a questionnaire to assess their level of education, current job responsibilities and experience with *in vitro* diagnostic test methods. The responses to this questionnaire were used to confirm that the participants were representative of typical operators in the intended use environment for near-patient testing.

To evaluate user perception of the SPOTFIRE System and SPOTFIRE R Panel, a Post-Instrument Setup Questionnaire and a Post-Study Questionnaire were administered to the operators who had been involved with instrument installation and/or performed testing to assess the ease of instrument assembly/set-up, usefulness of the SPOTFIRE Quick Guide, the ability of users to follow the instructions for Quality Control testing, the clarity of the onscreen instructions and to document any difficulties associated with the overall workflow.

Twelve individuals completed the Post-Instrument Setup Questionnaire, all of whom (100%) indicated that instrument installation was either “Easy” or “Very Easy” and reported no problems in stacking modules on the control station and connecting the cables. Similarly, most users found quality control testing to be “Easy” or “Very Easy”

and none reported any specific difficulties related to operating the system. Six of the 12 participants (50%) did experience some difficulty in scanning the pouch barcode, but further analysis attributed this to the font size used for the Investigational Use Only (IUO) labels, which was subsequently increased for product manufacture.

Of 35 operators who participated in the Clinical and/or Precision/Near Cut-off Studies with the SPOTFIRE R Panel, 26 completed the Post-Study Questionnaire. All (100%) reported using the Panel Quick Guide prior to running their first test and most referred to these instructions again at some point during the studies. Eight of 25 operators who answered the question (32.0%), indicated that they required help the first time they used the SPOTFIRE System, although in three cases, this was merely to confirm that that they were performing the procedure correctly. None of the operators contacted BIOFIRE for additional assistance to perform their first test, suggesting that they were able to complete the task following further review of the Panel Quick Guide and/or consultation with other study participants. In addition, all but one of the operators indicated that the Quick Panel Guide was "Easy" or Very Easy" to read and understand, and all of the operators reported that it was either "Easy" or "Very Easy" to prepare samples for testing.

Overall, the operators reported that the SPOTFIRE R Panel was easy to use and that the training materials provided (System Setup and Panel Quick Guides) were adequate to perform the test without additional instruction.

M. Labeling for Waived Devices

The labeling consists of:

1. SPOTFIRE R Panel Instructions For Use
2. SPOTFIRE R Panel Quick Guide
3. BIOFIRE SPOTFIRE System Operator's Manual
4. BIOFIRE SPOTFIRE System Setup Quick Guide

2. <u>The following elements are appropriately present:</u>
   - The SPOTFIRE System Operator's Manual specifies the environmental operating conditions under which testing may be performed.
   - The SPOTFIRE R Panel Quick Guide and SPOTFIRE System Setup Quick Guide are clear and easy to understand.
   - The SPOTFIRE R Panel Instructions For Use and Panel Quick Guide identify the test as CLIA Waived.
   - The SPOTFIRE R Panel Instructions For Use
     - Indicate that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test.
     - Include step-by-step instructions for performing the test.
     - Include safety considerations applicable for untrained users.
     - Specify the actions to be taken if an invalid test result is obtained.
     - Include a summary of the studies performed to support CLIA Waiver.

    - Include appropriate warnings and/or limitations pertaining to clinical interpretation of test results.
    - Include recommendations for Quality Control testing including the source of appropriate control materials and the frequency of testing.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

N. Benefit-Risk Considerations

Not applicable

O. Conclusion:

The submitted information in this CLIA waiver application supports a CLIA waiver approval decision.