CLIA Waiver by Application Approval Determination

Decision Summary

A. Document Number

CW230007

B. Parent Document Number

K230719

C. CLIA Waiver Type:

CLIA Waiver by Application

D. Applicant

BIOFIRE Diagnostics

E. Proprietary and Established Names

BIOFIRE SPOTFIRE Respiratory (R) Panel Mini for use with the BIOFIRE SPOTFIRE System

F. Measurand (analyte)

The BIOFIRE SPOTFIRE Respiratory (R) Panel Mini detects and identifies nucleic acids from the following pathogens: Coronavirus SARS-CoV-2, Human rhinovirus, Influenza A virus, Influenza B virus and Respiratory syncytial virus.

G. Sample Type(s)

Nasopharyngeal swabs

H. Type of Test

The BIOFIRE SPOTFIRE R Panel Mini 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 nucleic acids in nasopharyngeal swab (NPS) specimens obtained from individuals with signs and symptoms of respiratory tract infection including COVID-19.

Note: The BIOFIRE SPOTFIRE R Panel Mini is an identical product in terms of reagent formulation, form factor and instrument platform to the BIOFIRE SPOTFIRE Respiratory R Panel (K213954/CW210006) but with modified software

that reports five of the 15 targets found on the SPOTFIRE R Panel. The SPOTFIRE R Panel Mini maintains the same design features as the SPOTFIRE R panel and is likewise intended to be used in CLIA-waived environments.

I. Test System Description

1. Overview

The BIOFIRE SPOTFIRE R Panel Mini is a multiplexed nucleic acid-amplification-based test that is intended to detect, identify and differentiate various respiratory viral pathogens in nasopharyngeal swab (NPS) specimens from individuals suspected of respiratory tract infection, including COVID-19. The SPOTFIRE R Panel Mini 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 Mini 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 Mini Software is required to perform the testing with the SPOTFIRE R Panel Mini.

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

- BIOFIRE SPOTFIRE R Panel Mini 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 Mini 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 Mini pouch from its vacuum packaging and places it into the Pouch Loading Station. The SPOTFIRE R Panel Mini pouch is a closed system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple respiratory pathogens within a single NPS specimen. The user hydrates 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 air filters, 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)]>. 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 Mini 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 Mini 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

The Flex Studies described below were performed with the BIOFIRE SPOTFIRE R Panel using a subset of representative analytes that include enveloped RNA viruses which share similarities with the analytes targeted by the BIOFIRE SPOTFIRE R Panel Mini. As such, the Flex Studies performed with the BIOFIRE SPOTFIRE R Panel are also considered acceptable to support the robustness and ease-of use of the BIOFIRE

SPOTFIRE R Panel Mini. As applicable, reference to the BIOFIRE SPOTFIRE R Panel in the text encompasses the BIOFIRE SPOTFIRE R Panel Mini.

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 bacterial 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 obtained 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 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

Specification	Test Condition	Fail Safe/ Failure Alert	Agreement		Potential for Erroneous Results
			Negative	Positive	
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				
	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	Removal and re-addition of a module during a run				

Specification	Test Condition	Fail Safe/ Failure Alert	Agreement		Potential for Erroneous Results
			Negative	Positive	
modules	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
	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 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. 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 produce 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.

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

1. Comparison Study

a. Study Design

i. Study Sites and Duration

In the prospective clinical evaluation of the SPOTFIRE R Panel Mini, 1120 nasopharyngeal swab (NPS) specimens were collected from consented volunteers or obtained as residual, leftover specimens from subjects of all ages and tested at four study sites across the United States and one site in the United Kingdom over approximately seven months (December 2020 to June 2021).

ii. Operators

Prospective Clinical Study

A total of 29 operators participated in the Prospective Clinical Study, with between 1 and 15 operators per site. 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 Mini 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, 22 (75.9%) processed at least 5 nasopharyngeal samples that were positive

for one or more of the SPOTFIRE R Panel Mini analytes, as determined by the applicable comparator methods.

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.

iii. Instructions for Use

Operators who participated in evaluating the clinical performance of the SPOTFIRE R Panel Mini did not receive any training on how to perform the assay and were instructed to refer solely to the SPOTFIRE R Panel Mini 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

Prospectively Collected Specimens
The clinical performance (encompassing both accuracy and ease of use) of the SPOTFIRE R Panel Mini was established during a prospective multi-center study that was further supplemented with archived specimens (see below). Five geographically distinct urgent care or emergency department study sites representative of the intended use setting (four in the US and one in the UK) participated in these studies from December 2020 to June 2021. All SPOTFIRE R Panel Mini testing was performed according to the manufacturer's instructions by minimally trained operators. No hands-on training was provided to the SPOTFIRE R Panel Mini test operators; rather, training was limited to written materials (i.e., quick reference guides) that were intended to be included with the SPOTFIRE System. The inclusion and exclusion criteria for the study are summarized in **Table 7**.

Table 7. 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 NPS specimens were enrolled from consented volunteers or obtained as residual leftover specimens from subjects of all ages for the prospective clinical study; 95 of these NPS specimens were excluded for reasons listed in **Table 8**. The most common reason for specimen exclusion was that a valid SPOTFIRE R Panel Mini test result was not obtained. The final data set consisted of 1120 NPS specimens. Across the five study sites, 259 NPS specimens were initially collected and immediately frozen for later testing at the source study site. The remaining 861 NPS specimens were collected and tested fresh (without freezing). No difference in performance was observed when fresh and frozen specimen results were compared. Therefore, the data collected from 259 valid frozen specimens are combined with data from the valid 861 fresh specimens for all analyses.

Table 8. 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 Mini 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 Mini result	38
Specimen not received for comparator testing	29
Use of expired SPOTFIRE R Panel Mini 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 on the SPOTFIRE R Panel Mini were of low prevalence during the prospective study and were not encountered in large enough numbers to demonstrate system performance. To supplement the results of the prospective clinical study, an evaluation of preselected archived retrospective NPS specimens was performed.

A total of 562 frozen archived NPS specimens were obtained from 11 external laboratories world-wide and retrospectively tested at the four US clinical sites. Of these, 542 NPS specimens had valid results that were included in performance analysis.

vi. Comparative Method (CM)

A description of each of the comparator methods used to establish the performance of the SPOTFIRE R Panel Mini is provided in **Table 9**. The analyte composition of the archived specimens was confirmed using the same comparator methods as the prospective study.

Table 9. Comparator methods used with prospectively collected and archived clinical specimens

SPOTFIRE R Panel Mini Assay	FDA-Cleared Comparator Method
Human Rhinovirus	Multi-Analyte Panel-1
Influenza A Virus	
Respiratory Syncytial Virus	
Coronavirus SARS-CoV-2 [1]	Multi-Analyte Panel-2
Influenza B Virus [2]	

[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

b. *Results and Analysis*

i. Statistical Analysis of Comparison Study Results

Prospective Clinical Study

The performance of the SPOTFIRE R Panel Mini was evaluated by comparing the test results with those from FDA-cleared multiplexed respiratory pathogen panels. The performance for the prospective study is summarized in **Table 10**. Positive percent agreement (PPA) for each analyte was calculated as 100% × (TP / (TP + FN)). True positive (TP) indicates that both the SPOTFIRE R Panel Mini and the comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the SPOTFIRE R Panel Mini was negative while the comparator result was positive. Negative percent agreement (NPA) was calculated as 100% × (TN / TN + FP)). True negative (TN) indicates that both the SPOTFIRE R Panel Mini and the comparator method had negative results, and false positive (FP) indicates that the SPOTFIRE R Panel Mini was positive while the comparator method was negative. The exact binomial two-sided 95% confidence interval (95%CI) was calculated. Investigations of discrepant results are summarized in the footnotes.

Table 10. SPOTFIRE R Panel Mini Clinical Performance Summary with NPS Specimens

SPOTFIRE R Panel Mini Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Coronavirus SARS-CoV-2[a]	71/73	97.3	90.5-99.2%	1031/1037	99.4	98.7-99.7%
Human rhinovirus[b]	345/348	99.1	97.5-99.7%	695/767	90.6	88.3-92.5%
Influenza A virus	0/0	-	-	1115/1115	100	99.7-100%
Influenza B virus	0/0	-	-	1110/1110	100	99.7-100%
Respiratory syncytial virus[c]	26/27	96.3	81.7-99.3%	1086/1088	99.8	99.3-99.9%

[a] SARS-CoV-2 was detected in 1/2 FN specimens upon SPOTFIRE R Panel Mini retest. SARS-CoV-2 was detected in 2/6 FP specimens using an additional molecular method.

[b] Human rhinovirus was detected in 1/3 FN specimens upon SPOTFIRE R Panel Mini retest. Human rhinovirus was detected in 48/72 FP specimens using an additional molecular method.

[c] Respiratory syncytial virus was detected in the single FN specimen upon SPOTFIRE R Panel Mini retest. Respiratory syncytial virus was detected in 1/2 FP specimens using an additional molecular method.

The overall success rate for initial specimen tests was 96.7% (1158/1198). Eight tests (8/1198; 0.7%) did not complete on the initial attempt, resulting in an instrument success rate of 99.3% (1190/1198). Retests were not possible due to insufficient specimen volume. Of the 1190 tests that successfully produced a completed run on the initial test, 1158 (97.3%) had valid internal process controls. This represents a 97.3% (1158/1190) success rate for internal process controls in completed runs in the initial specimen tests.

Archived Specimens

The specimens were randomized such that the users performing both the confirmation and the SPOTFIRE R Panel Mini testing were blinded to the expected test result. The results of the SPOTFIRE R Panel Mini performance for these archived specimens is shown in **Table 11**.

Table 11. SPOTFIRE R Panel Mini Archived Performance Summary with NPS Specimens

SPOTFIRE R Panel Mini Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Coronavirus SARS-CoV-2	0/0	-	-	0/0	-	-
Human rhinovirus[a]	29/30	96.7	83.3-99.4%	439/454	96.7	94.6-98.0%
Influenza A virus[b]	58/59	98.3	91.0-99.7%	423/423	100	99.1-100%
Influenza B virus	30/30	100	88.6-100%	28/28	100	87.9-100%
Respiratory syncytial virus[c]	37/37	100	90.6-100%	440/447	98.4	96.8-99.2%

[a] The single FN specimen was unable to be investigated. Human rhinovirus was detected in 4/14 FP specimens during discrepancy investigation using an additional molecular method; one additional FP specimen was unable to be investigated.
[b] Influenza A virus was detected in the single FN specimen by standard of care.
[c] Respiratory syncytial virus was detected in 4/6 FP specimens during discrepancy investigation using an additional molecular method; one additional FP specimen was unable to be investigated.

Precision/Near Cut-Off Study

A near-LoD/ reproducibility evaluation was performed to demonstrate that the SPOTFIRE R Panel Mini could provide accurate results for weak-positive and negative samples when used by minimally trained operators. Contrived samples were tested by naïve operators at three of the prospective clinical study sites and additionally on three unique

SPOTFIRE Systems at BioFire Diagnostics (BioFire) by trained BioFire personnel. The contrived samples contained combinations of SPOTFIRE R Panel Mini analytes prepared at or near (1× to 3×) the LoD, as well as negative samples.

For testing performed at clinical sites, samples were tested over five testing events (non-consecutive days) by two operators during the course of their normal workday routine. Each site was equipped with a single SPOTFIRE System. Testing at all three sites was performed with a single reagent lot. For each testing event, each operator ran two replicate pouches for a total of 20 replicates per site and 60 total replicates across all three sites (2 operators x 2 samples x 3 sites x 5 days = 60 replicates).

For testing performed at BioFire, samples were tested over five consecutive days, by two operators per system, using three different reagent lots. On each day of testing, the two operators each tested three replicates on each system for a total of 30 replicates per system and 90 total replicates overall (2 operators x 3 samples x 3 instruments x 5 days = 90 replicates).

When combined across all sites, each panel member was tested in a total of 150 replicates by at least 12 different operators across six different SpotFire Systems.

A summary of results (percent (%) agreement with the expected positive or negative result) for each analyte (by site and system) is provided in **Table 12**. The SPOTFIRE R Panel Mini reported the expected positive results for panel analytes with 98.9% -100% of samples and the expected negative results for all analytes with 100% of samples. Comparison of the positive percent agreement between user groups (99.8% for trained operators at BIOFIRE versus 99.2% for minimally trained operators) demonstrates that the accuracy of the SPOTFIRE R Panel Mini is not dependent upon the specific expertise of the user.

Table 12. Reproducibility of Results with the SPOTFIRE R Panel Mini and BIOFIRE SPOTFIRE System

Analyte Isolate (Source ID)		Concentration Tested (test level)	Expected Result	SpotFire System testing								All Sites /Systems [95% Confidence Interval]
				BioFire Dx				Clinical				
				System A	System B	System C	Total	Site 1	Site 2	Site 3	Total	
Coronavirus SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2 (ATCC VR-1986HK)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600** **100%** [99.4-100%]
		2.5E+02 copies/mL (1× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90** **(100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60** **(100%)**	**150/150** **100%** [97.6-100%]
Human rhinovirus Enterovirus D68 US/MO/14-18947 (ATCC VR-1823)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600** **100%** [99.4-100%]
		1.1E+01 $TCID_{50}$/mL (1× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90** **(100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60** **(100%)**	**150/150** **100%** [97.6-100%]
Influenza A virus	**No Analyte**		**Negative**	90/90 (100%)	90/90 (100%)	90/90 (100%)	270/270 (100%)	60/60 (100%)	60/60 (100%)	60/60 (100%)	180/180 (100%)	**450/450** **100%** [99.2-100%]
	Influenza A H1N1pdm (ZeptoMetrix 0810538CF)	2.5E+00 $TCID_{50}$/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90** **(100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60** **(100%)**	**150/150** **100%** [97.6-100%]
	Influenza A H3N2 (ZeptoMetrix 0810526CF)	2.6E+00 $TCID_{50}$/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90** **(100%)**	19/20 (95.0%)	19/20 (95.0%)	20/20 (100%)	**58/60** **(96.7%)**	**148/150** **98.7%** [95.3-99.8%]
Influenza B virus (ZeptoMetrix 0810037CF)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600** **100%** [99.4-100%]
		9.9E-02 $TCID_{50}$/mL (3× LoD)	**Positive**	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	**89/90** **(98.9%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60** **(100%)**	**149/150** **99.3%** [96.3-100%]
Respiratory syncytial virus (ZeptoMetrix 0810040ACF)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600** **100%** [99.4-100%]
		6.2E-02 $TCID_{50}$/mL (1× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90** **(100%)**	20/20 (100%)	19/20 (95.0%)	20/20 (100%)	**59/60** **(98.3%)**	**149/150** **99.3%** [96.3-100%]
Total positive agreement (%) by system/user group				179/180 99.4%	180/180 100%	180/180 100%	**539/540** **99.8%**	119/120 99.2%	118/120 98.3%	120/120 100%	**357/360** **99.2%**	**896/900** **99.6%** [98.9-99.9%]
Overall positive agreement (%) [95% Confidence Interval]												

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. Because the workflow for the BIOFIRE SPOTFIRE R Panel Mini is the same as that for the BIOFIRE SPOTFIRE R Panel, the feedback obtained from the Operator Questionnaire is applicable to both test systems.

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

The following elements are appropriately present:

- The SPOTFIRE System Operator's Manual specifies the environmental operating conditions under which testing may be performed.
- The SPOTFIRE R Panel Mini Quick Guide and SPOTFIRE System Setup Quick Guide are clear and easy to understand.
- The SPOTFIRE R Panel Mini Instructions For Use and Panel Quick Guide identify the test as CLIA Waived.
- The SPOTFIRE R Panel Mini 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.