CLIA Waiver by Application Approval Determination

Decision Summary

A. Document Number

CW240014

B. Parent Document Number

K241194

C. CLIA Waiver Type:

CLIA Waiver by Application

D. Applicant

BIOFIRE Diagnostics

E. Proprietary and Established Names

BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini for use with the BIOFIRE SPOTFIRE System

F. Measurand (analyte)

The BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) Panel Mini can be performed either in the BIOFIRE SPOTFIRE Respiratory (R) Panel Mini or The BIOFIRE SPOTFIRE Sore Throat (ST) Panel Mini configuration.

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.

The BIOFIRE SPOTFIRE Sore Throat (ST) Panel Mini detects and identifies nucleic acids from the following pathogens: Human rhinovirus, Influenza A virus, Influenza B virus, Respiratory syncytial virus, and *Streptococcus pyogenes* (group A Strep).

G. Sample Type(s)

Nasopharyngeal swabs for the BIOFIRE SPOTFIRE Respiratory (R) Panel Mini or

Throat swab for the BIOFIRE SPOTFIRE Sore Throat (ST) Panel Mini

H. Type of Test

The BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) 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 and bacterial nucleic acids in nasopharyngeal swab (NPS) specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19; (Respiratory menu) or in throat swab (TS) specimens from individuals with signs and symptoms of pharyngitis (Sore Throat menu).

Note: The BIOFIRE SPOTFIRE R/ST Panel Mini is an identical product in terms of reagent formulation, form factor and instrument platform to the BIOFIRE SPOTFIRE Respiratory R/ST Panel (K232954/CW230018) but with modified software that reports five of the 15 targets found on the SPOTFIRE Respiratory R/ST Panel. The SPOTFIRE R/ST Panel Mini maintains the same design features as the SPOTFIRE R/ST panel and is likewise intended to be used in CLIA-waived environments.

I. Test System Description

1. Overview

The BIOFIRE SPOTFIRE Respiratory/Sore Throat (R/ST) 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 and bacterial nucleic acids in nasopharyngeal swab (NPS) specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19; (Respiratory menu) or in throat swab (TS) specimens from individuals with signs and symptoms of pharyngitis (Sore Throat menu). The SPOTFIRE R/ST 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/ST 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/ST Panel Mini Software is required to perform the testing with the SPOTFIRE R/ST Panel Mini.

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

- BIOFIRE SPOTFIRE R/ST 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/ST Panel Mini requires either a nasopharyngeal swab (NPS) specimen (respiratory menu) or throat swab (TS) specimen (sore throat menu) to be collected according to standard procedures and immediately placed in either 3 mL or 1mL of compatible transport medium, respectively. 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/ST Panel Mini pouch from its vacuum packaging and places it into the Pouch Loading Station. The SPOTFIRE R/ST 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 to isolate, amplify, and detect nucleic acid from multiple respiratory pathogens within a single NPS or TS 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 or throat 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)]>. In rare cases, Influenza A may be reported as <UNCERTAIN: Influenza A> with follow-on actions found in the Quick Reference Guide. 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/ST 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/ST 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

Process Monitored	Diagnostic Self-Test		Message/Run Status
	Power-on	Run-time	
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 below.

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

Fail-safe or Failure Alert Mechanism	Description	Failure Modes Prevented
	• 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

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

Flex Studies were performed to evaluate the robustness of the BIOFIRE SPOTFIRE System and SPOTFIRE R/ST 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 or throat swab specimen matrices 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 and were tested using both nasopharyngeal and throat swab specimens.

The composition of the positive samples used to verify system performance under normal conditions (controls) and as test materials in subsequent Flex Studies is described in **Table 3**. The analytes were selected to include representative Gram-positive and Gram-negative

bacterial species and different types of viruses (DNA, RNA, enveloped, non-enveloped) that are detected by the BIOFIRE SPOTFIRE R/ST Panel.

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

Sample Type	Analyte	Description	Strain	Source ID	Per mL
Nasopharyngeal Swab	Parainfluenza Virus	Enveloped RNA virus	2	ZeptoMetrix 08100015CF	42 $TCID_{50}$
	Human Metapneumovirus	Enveloped RNA virus	B1-3 Peru2-2002	ZeptoMetrix 0810156CF	0.75 $TCID_{50}$
	Adenovirus	Non-enveloped DNA virus	B Serotype 3	ZeptoMetrix 0810062CF	2.4 $TCID_{50}$
	Bordetella parapertussis	Gram negative bacterium	E595	ZeptoMetrix 0801462	120 CFU
	Mycoplasma pneumoniae	Intracellular bacterium	M129	ZeptoMetrix 0810579	30 CCU
Throat Swab	Influenza A H3N2	Enveloped RNA virus	Hong Kong/480/114	ZeptoMetrix 0810526CF	2.6 $TCID_{50}$
	Coronavirus (seasonal)	Enveloped RNA virus	NL63	ZeptoMetrix 0810228CF	7.5×10^{-3} $TCID_{50}$
	Human Rhinovirus	Non-enveloped RNA virus	1A	ZeptoMetrix 0810012CFN	0.63 $TCID_{50}$
	Streptococcus pyogenes	Gram positive bacterium	SF-130 T1	ATCC 12344	1.4×10^{3} cells

[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 with both nasopharyngeal and throat swab specimens. 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 workflow for the BIOFIRE SPOTFIRE R/ST Panel was validated in the Clinical Study with naïve operators and performance was shown to be acceptable.

Selection of Incorrect Specimen Type

In addition, to the above two conditions that could lead to incorrect results there is potential for the test operator to select the incorrect specimen type when choosing the test protocol to be performed on a given specimen. Even though the reagent pouch would be expected to function correctly in these circumstances, such a mistake would lead to incomplete reporting of results for all the analytes applicable to the chosen specimen type in addition to reporting of results for some analytes that are not appropriate to the specimen type being tested. To reduce the potential for such errors, the BIOFIRE SPOTFIRE user interface requires the operator to verify the specimen type prior to insertion of the reagent pouch into the test module. In the Prospective Clinical Study described in **Section (L)** and K232954, operators selected the correct specimen type 99.7% of the time (1991/1997 BIOFIRE SPOTFIRE R/ST Panel tests performed), indicating that the likelihood of selecting the incorrect specimen type is low. In addition, should such an error occur, the BIOFIRE SPOTFIRE R/ST Panel test report is generated automatically upon completion of a run and clearly displays the panel type (Respiratory or Sore Throat) that was selected, together with the results for each applicable analyte. If the incorrect specimen type was selected, in most cases, sufficient specimen volume should remain for retesting, thereby limiting the effect on patient management.

Overall, the Flex Studies and supporting data from the Prospective Clinical Study demonstrated that the BIOFIRE SPOTFIRE R/ST 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 with the BIOFIRE SPOTFIRE R/ST Panel

Specification	Test Condition	Fail Safe/ Failure Alert	Agreement				Potential for Erroneous Results
			Negative		Positive		
			aNS	aTS	aNS	aTS	
Reagent shelf-life	Attempt to use expired reagent kit/pouches	Yes	Lockout activated		N/A	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	3/3	6/6	6/6	No
	2-8 °C	No	3/3	3/3	11/12 [1]	6/6	No
	≥ 40 °C	No	3/3	3/3	6/6	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	3/3	6/6	11/12 [2]	No
Pause 5 seconds after unscrewing the Sample	Alternating positive aNS and aTS samples tested	No	6/6	6/6	6/6	6/6	No

Specification	Test Condition	Fail Safe/ Failure Alert	Agreement				Potential for Erroneous Results
			Negative		Positive		
			aNS	aTS	aNS	aTS	
Injection Vial to prevent dripping	without the specified 5 second delay or between-sample cleaning						
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	3/3	6/6	6/6	No
	15 - 25 °C under UV light	Yes	3/3 Invalid	3/3 Invalid	N/A	N/A	No
	-20 °C	No	3/3	3/3	6/6	6/6	No
	2-8 °C	No	3/3	3/3	6/6	6/6	No
	40 °C	No	3/3	3/3	6/6	6/6	No
Reagent pouch is robust to normal handling	Pouch dropped after loading	No	3/3	3/3	6/6	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	3/3	**0/6**	**0/6**	**Yes [3]**
	100 µL ((33%)	No	3/3	3/3	6/6	6/6	No
	600 µL (200%)	No	3/3	3/3	6/6	11/12 [4]	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	3/3 Invalid	N/A	N/A	No
	100 µL (12.5%)	No	3/3	3/3	6/6	6/6	No
Operator inverts the Sample Injection Vial 3X to mix	Failure to mix or excessive mixing						
	No mixing	No	3/3	3/3	6/6	6/6	No
	5X inversion	No	3/3	3/3	6/6	6/6	No
	Vigorous shaking	No	3/3	3/3	6/6	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	3/3 Invalid	N/A	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	3/3 Invalid	N/A	N/A	No
	20-45 % of target hydration volume added by weight	Yes	3/3 Invalid	3/3 Invalid	N/A	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	3/3 Invalid	N/A	N/A	No
	12.5 - 60% target volume by weight	No	3/3	3/3	**4/6 [5]**	**3/6 [6]**	**Yes [3]**

Specification	Test Condition	Fail Safe/ Failure Alert	Agreement				Potential for Erroneous Results
			Negative		Positive		
			aNS	aTS	aNS	aTS	
Reagent pouch is single use	Attempt to re-use a spent pouch						
	On the same SPOTFIRE System	Yes	Lockout Activated		N/A	N/A	No
	On a different SPOTFIRE System	Yes	Instrument Error		N/A	N/A	No
Selection of sample type (nasopharyngeal swab or throat swab)	Selection of the incorrect swab type	No	3/3	3/3	6/6 [7]	6/6 [8]	**Results for some analytes missing or not applicable [3]**
Ability to reset System/module to clear errors	Inadvertent or deliberate misuse of the reset button	Yes [9]	3/3	3/3	6/6	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	3/3	6/6	6/6	No
	Active module	No	3/3	3/3	6/6	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	3/3	6/6	11/12 [10]	No
	Operation at a 20 ° angle from vertical	No	3/3	3/3	11/12 [11]	6/6	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	3/3	6/6	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	3/3	6/6	6/6	No
The SPOTFIRE System is robust to vibration	System operated near equipment generating high or low frequency vibration						
	Low frequency (1.040-2.00 mm/s; 0.043-0.079 g)	No	3/3	3/3	6/6	6/6	No
	High frequency (0.92-1.09 mm/s; 0.089 g)	No	3/3	3/3	6/6	6/6	No

aNS: artificial nasopharyngeal swab matrix; aTS: artificial throat swab matrix; 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; 6/6 additional replicates produced the expected results.

[2] 1/6 replicates reported negative for Influenza A-H3 on initial testing; 6/6 additional replicates produced the expected results.

[3] The workflow for the SPOTFIRE R/ST 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 erroneous results due to this failure mode is considered low.

[4] 1/6 replicates reported negative for Influenza A-H3 on initial testing; 6/6 additional replicates produced the expected results.

[5] 2/6 replicates reported negative for Human Metapneumovirus and Parainfluenza Virus.

[6] 3/6 replicates reported negative for Coronavirus (seasonal); 1/6 replicates reported negative for Human Rhinovirus/Enterovirus; 1/6 replicates reported negative for Influenza A-H3.

[7] Results not reported for *B. parapertussis* when Throat Swab selected as the sample type (this analyte is not included on the Sore Throat Panel).

[8] Results not reported for *S. pyogenes* when Nasopharyngeal Swab selected as the sample type (this analyte is not included on the Respiratory Panel).

[9] Reset not permitted while a run is in progress.

[10] 1/6 replicates reported negative for Influenza A-H3 on initial testing; 6/6 additional replicates produce the expected results.

[11] 1/6 replicates negative for Human Metapneumovirus on initial testing; 6/6 additional replicates produced the expected results.

Specimen Stability

Nasopharyngeal Swabs

Analytical studies to demonstrate the stability of the applicable BIOFIRE SPOTFIRE R/ST Panel analytes in nasopharyngeal swab matrix in viral transport medium (VTM) are documented under K213954. The data support the claimed storage conditions for nasopharyngeal swabs for use with the BIOFIRE SPOTFIRE R/ST Panel of up to 4 hours at 15-25 °C, 3 days at 2-8 °C or 30 days at ≤ -15 °C.

Throat Swabs

Analytical studies to demonstrate the stability of the applicable BIOFIRE SPOTFIRE R/ST Panel analytes in throat swab matrix in liquid Amies medium are documented under K232954/CW230018. Natural throat swab matrix in Amies medium was obtained from asymptomatic volunteers and was spiked with representative target analytes from the Sore Throat Panel at a concentration of 3X LoD and tested either immediately (baseline) or after storage under different conditions. Five different mixtures of target analytes were included in the study. Each was tested 10 times at each time point after storage under the applicable condition. All the analytes were shown to be stable under the conditions tested. The results of this study support the stability of the BIOFIRE SPOTFIRE R/ST Panel analytes in throat swab specimens in liquid Amies medium for up to 4 hours at 25 °C, 3 days at 2-8 °C or 30 days at ≤ -15 °C prior to testing.

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

1. Comparison Study

a) *Study Design*

i. *Study Sites and Duration*

The clinical performance (encompassing both accuracy and ease of use) of the SPOTFIRE R/ST Panel Mini was established during a prospective multi-center study that was further supplemented with archived and contrived specimens. Six geographically distinct urgent care or emergency department study sites representative of the intended use setting (five in the US and one in the UK) participated in these studies from December 2020 to September 2021 (NPS and TS specimens) and from September 2022 to May 2023 (TS specimens only). All SPOTFIRE R/ST Panel Mini testing was performed according to the manufacturer's instructions by operators with training and educational backgrounds representative of those in the CLIA-waived or near-patient testing setting. No hands-on training was provided to the SPOTFIRE R/ST Panel Mini test operators; rather, training was limited to written materials (i.e. quick reference guides) that were intended to be included with the BIOFIRE SPOTFIRE System.

A total of 1215 NPS specimens and 1165 TS 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 and 288 of these TS specimens were excluded. The most common reasons for specimen exclusion were that the SPOTFIRE R/ST Panel Mini pouch was later determined to be expired or the specimen was unable to be tested within the designated timeframe. The final data set consisted of 1120 NPS specimens and 877 TS specimens. Across the six study sites, 352 specimens (259 NPS specimens and 93 TS specimens) were initially collected and immediately frozen for later testing at the source study site. The remaining 1645 specimens (861 NPS specimens and 784 TS 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 352 valid frozen specimens are combined with data from the valid 1645 fresh specimens for all analyses.

ii. *Operators*

Prospective Clinical Study

A total of 42 operators participated in the Prospective Clinical Study, with between 1 and 17 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.

All (100%) 42 participating operators processed at least 1 throat swab specimen while 29 (69.0%) also processed at least 1 nasopharyngeal swab. Twenty-four of the 42 operators (57.1%) processed at least 5 positive and 5 negative nasopharyngeal swabs specimens, and 31 (73.8%) processed at least 5 positive and 5 negative throat swab specimens, 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 was easy to perform using the System Setup and Panel Quick Guides, without the need for additional training materials.

Archived Specimen Testing
Seventeen of the original 42 operators performed testing of the archived specimens at four U.S. clinical sites used in the Prospective Clinical Study. All sites and operators tested multiple nasopharyngeal specimens. Four operators at two sites processed both nasopharyngeal and throat swab specimens.

Contrived Sample Testing
Three of the four sites that performed archived specimen testing also assayed contrived samples using a total of six operators.

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

iv. *Subject and Specimen Enrollment*

Prospectively Collected Specimens
A Clinical Study with prospectively collected specimens was conducted at six sites (5 U.S. and 1 outside of the 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 BIOFIRE SPOTFIRE R/ST Panel. The specimens included in the study were either collected under Informed Consent, or with parental permission and individual assent (if appropriate) 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 K232954. Specimen enrollment took place over two periods: both nasopharyngeal and throat swab specimens were enrolled between December 2020 and September 2021 and enrollment of throat swab specimens was continued from September 2022 to May 2023.

Initial enrollment in the Prospective Clinical Study included 1215 nasopharyngeal and 1165 throat swabs. Study enrollment criteria can be found in K232954. Of these, 95 nasopharyngeal swabs and 288 throat swabs were excluded from the analysis of performance. The most common reasons for specimen exclusion were that the SPOTFIRE R/ST Panel Mini pouch was later determined to be expired or the specimen was unable to be tested within the designated timeframe. All nasopharyngeal swabs included in the analysis of performance were collected in viral transport medium (VTM), whereas all throat swabs were collected in liquid Amies medium. The final data set consisted of 1120 NPS specimens and 877 TS specimens. Across the six study sites, 352 specimens (259 NPS specimens and 93 TS specimens) were initially collected and immediately frozen for later testing at the source study site. The

remaining 1645 specimens (861 NPS specimens and 784 TS specimens) were collected and tested fresh (without freezing).

Archived and Contrived Specimens

Several analytes on the SPOTFIRE R/ST Panel Mini were of low prevalence during the prospective study and were not encountered in large enough numbers to adequately demonstrate system performance. To supplement the results of the prospective clinical study, an evaluation of preselected archived retrospective NPS and TS specimens was performed. Archived and contrived sample testing occurred over a 3-4 month period during the respiratory season as to integrate testing into normal everyday workflow alongside prospectively collected samples.

A total of 562 archived nasopharyngeal swabs and 136 throat swabs was obtained. Of these, 542 nasopharyngeal swabs and 128 throat swabs. The specimens were randomized such that the users performing both the confirmation and the SPOTFIRE R/ST Panel Mini testing were blinded to the expected test result. Samples were tested at four of the U.S. clinical sites included in the prospective study.

Several analytes were not observed in TS specimens in sufficient numbers to demonstrate SPOTFIRE R/ST Panel Mini performance in the prospective and archived specimen studies. Therefore, contrived specimens (N=431; at least 50 for each analyte) were made from unique, analyte-negative clinical TS specimens that were spiked with a variety of different isolates/strains for each organism at concentrations that spanned the detection range of each assay. At least half (50%) of the contrived specimens had analyte concentrations at 2x LoD. Contrived specimens were randomized and blinded, along with 29 negative (unspiked) specimens such that the analyte status of each specimen was unknown to the users performing the testing. The blinded contrived specimens were distributed to three of the prospective clinical study sites for testing.

v. *Comparator Method*
A description of the comparator methods used to establish the performance of the BIOFIRE SPOTFIRE R/ST Panel is provided in **Table 5**.

Table 5. Comparator methods used to characterize prospectively collected and archived clinical specimens

Analyte	BIOFIRE SPOTFIRE R/ST Panel		Comparator Method
	Respiratory	Sore Throat	
Human Rhinovirus [1]	X	X	FDA-cleared Multi-Analyte Panel-1
Influenza A Virus	X	X	
Respiratory Syncytial Virus	X	X	
Coronavirus SARS-CoV-2 [2]	X		FDA-cleared Multi-Analyte Panel-2
Influenza B Virus [3]	X	X	

Analyte	BIOFIRE SPOTFIRE R/ST Panel		Comparator Method
	Respiratory	Sore Throat	
Streptococcus pyogenes (Group A *Streptococcus*)		X	*Method 1:* Culture for b-hemolytic *Streptococcus*, followed by species identification by PCR of 16S rRNA with bidirectional sequencing performed on the culture isolate [4] *Method 2:* 2 PCR assays with bidirectional sequencing

[1] Human enterovirus is reported as Human Rhinovirus on the BIOFIRE SPOTFRE R/ST Panel Mini
[2] Not detected by Multi-Analyte Panel-1 comparator.
[3] 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.
[4] Culture was not performed for archived specimens.

b) *Results and Analysis*

Nasopharyngeal Swabs
A summary of the results from testing prospectively collected and archived nasopharyngeal swabs is shown in **Table 6** and **7**. Positive Percent Agreement (PPA) ranged from 96.3-100% for prospectively collected specimens and from 96.73-100% 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 with prospectively collected specimens. However, additional testing provided evidence for the presence of Human Rhinovirus/Enterovirus in 54/72 prospectively collected specimens (75.0%) with discordant positive BIOFIRE SPOTFIRE R/ST Panel results and therefore the low NPA with this analyte in the prospective study was considered acceptable.

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

Table 6. SPOTFIRE R/ST Panel Mini Prospective Clinical Performance Summary for NPS Specimens – Respiratory Menu

	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Viruses						
Coronavirus SARS-CoV-2[1]	71/73	97.3	90.5-99.2%	1031/1037	99.4	98.7-99.7%
Human rhinovirus[2]	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[3]	26/27	96.3	81.7-99.3%	1086/1088	99.8	99.3-99.9%

95% CI: 2-sided 95% score confidence interval; TP: True Positive; FP: False-Positive; False-Negative; True Negative (all as determined with respect to the comparator)

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

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

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

Table 7. SPOTFIRE R/ST Panel Mini Archived Performance Summary for NPS Specimens – Respiratory Menu

	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
Coronavirus SARS-CoV-2	0/0	-	-	0/0	-	-
Human rhinovirus[1]	29/30	96.7	83.3-99.4%	439/454	96.7	94.6-98.0%
Influenza A virus[2]	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[3]	37/37	100	90.6-100%	440/447	98.4	96.8-99.2%

95% CI: 2-sided 95% score confidence interval; TP: True Positive; FP: False-Positive; False-Negative; True Negative (all as determined with respect to the comparator)

[1] 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.

[2] Influenza A virus was detected in the single FN specimen by standard of care.

[3] 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.

Throat Swabs

The performance of the BIOFIRE SPOTFIRE R/ST Panel with prospectively collected and archived throat swab specimens is summarized in **Table 8** and **9**. Positive Percent Agreement (PPA) ranged from 87.5-100% for prospectively collected specimens and from 97.5-100% for archived specimens, depending on the analyte, whereas Negative Percent Agreement (NPA) ranged from 93.5-100% for prospectively collected specimens and from 96.5-100% for archived specimens. The only analyte for which PPA was lower was for RSV, however, this was due to low prevalence during clinical studies and a limited number of prospective and achieved samples. Additional testing of 25 contrived positive RSV samples at the 2x LoD level, 25 positive samples at higher LoD concentration s, and 381

negative samples demonstrated a PPA of 98% (95% CI 89.5-99.6%) and 100% NPA (99.0-100%).

Also, based on the observed clinical sensitivity of the BIOFIRE SPOTFIRE R/ST Panel Mini for the detection of *S. pyogenes* (Group A *Streptococcus*) in throat swab specimens, culture confirmation of negative BIOFIRE SPOTFIRE R/ST Panel results for this analyte is not required. However, the following Limitation is included in the BIOFIRE SPOTFIRE R/ST Panel Mini device labeling:

> *"Additional follow-up testing by culture is required if the SPOTFIRE R/ST Panel Mini Streptococcus pyogenes assay result is negative and clinical symptoms persist, or in the event of an outbreak of acute rheumatic fever (ARF)."*

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

Table 8. SPOTFIRE R/ST Panel Mini Prospective Clinical Performance Summary for TS Specimens – Sore Throat Menu[1]

		Sensitivity/PPA			Specificity/NPA		
		TP/(TP + FN)	**%**	**95%CI**	**TN/(TN + FP)**	**%**	**95%CI**
Viruses							
Human rhinovirus[2]		202/213	94.8	91.0-97.1%	619/662	93.5	91.4-95.1%
Influenza A virus		35/35	100	90.1-100%	840/840	100	99.5-100%
Influenza B virus		4/4	100	51.0-100%	872/872	100	99.6-100%
Respiratory syncytial virus[3]		21/24	87.5	69.0-95.7%	849/851	99.8	99.1-99.9%
Bacteria							
Streptococcus pyogenes (group A Strep)	PCR[4]	209/217	96.3	92.9-98.1%	654/660	99.1	98.0-99.6%
	Culture[5]	174/177	98.3	95.1-99.4%	654/692	94.5	92.6-96.0%

95% CI: 2-sided 95% score confidence interval; TP: True Positive; FP: False-Positive; False-Negative; True Negative (all as determined with respect to the comparator)

1 The performance measures of sensitivity and specificity only refer to the *Streptococcus* analyte for which culture was used as the reference method. Performance measures of PPA and NPA refer to all other analytes, for which molecular assays were used as comparator methods.

2 Human rhinovirus was detected in 7/11 FN specimens using an additional molecular method. Human rhinovirus was detected in 14/43 FP specimens using an additional molecular method.

3 Respiratory syncytial virus was detected in all three FN specimens upon SPOTFIRE R/ST Panel Mini retest. Respiratory syncytial virus was detected in 1/2 FP specimens using an additional molecular method.

4 *S. pyogenes* was detected in 7/8 FN specimens during discrepancy investigation: four using an additional molecular method and three upon SPOTFIRE R/ST Panel Mini retest. *S. pyogenes* was detected in 2/6 FP specimens using an additional molecular method.

5 *S. pyogenes* was detected in all three FN specimens during discrepancy investigation: one using an additional molecular method and two upon SPOTFIRE R/ST Panel Mini retest. *S. pyogenes* was detected in 34/38 FP specimens using an additional molecular method.

Table 9. SPOTFIRE R/ST Panel Mini Archived Performance Summary for TS Specimens – Sore Throat Menu

	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
Human rhinovirus[1]	2/2	100	34.2-100%	55/57	96.5	88.1-99.0%
Influenza A virus	11/11	100	74.1-100%	44/44	100	92.0-100%
Influenza B virus	20/20	100	83.9-100%	0/0	-	-
Respiratory syncytial virus[2]	2/2	100	34.2-100%	56/57	98.2	90.7-99.7%
Bacteria						
Streptococcus pyogenes (group A Strep)[3]	38/39	97.4	86.8-99.5%	10/10	100	72.2-100%

95% CI: 2-sided 95% score confidence interval; TP: True Positive; FP: False-Positive; False-Negative; True Negative (all as determined with respect to the comparator)

[1] Human rhinovirus both FP specimens were unable to be investigated.

[2] Respiratory syncytial virus was detected in the single FP specimen by standard of care.

[3] *Streptococcus pyogenes* was detected in the single FN by standard of care.

2. Device Performance with Analyte Concentrations Near the Cutoff

A near-LoD evaluation was combined with reproducibility and was performed to demonstrate that the SPOTFIRE R/ST Panel Mini could reproducibly provide accurate results for weak-positive and negative samples when used by minimally trained operators. Contrived samples were tested 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/ST Panel Mini analytes prepared at or near (1-3×) the LoD. Studies were conducted at three external clinical laboratories and one internal. External sites used a single reagent lot over 5 non-consecutive days with 2 operators running two replicate pouches per day per testing site for a total of 20 replicates per site and 60 replicates across all external sites. (2 operators x 2 samples x 3 sites x 5 days = 60 replicates).

Internal laboratory testing at BioFire Dx were tested over five consecutive days, by two operators per system, using three different reagent lots. 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 across all systems (2 operators x 3 samples x 3 instruments x 5 days = 90 replicates). When combined, each analyte was tested in a total of 150 replicates by at least 12 different operators across six different SPOTFIRE Systems.

The SPOTFIRE R/ST Panel Mini reported the expected positive results for panel analytes in 98% -100% of samples and the expected negative results for all analytes in 100% of samples (**Table 10)**. Comparison of the positive percent agreement between user groups (99.8% for trained operators at BioFire versus 99.0% for minimally trained operators) demonstrates that the accuracy of the SPOTFIRE R/ST Panel Mini is not dependent upon the specific expertise of the user.

Table 10. Near LoD/ Reproducibility of Results for SPOTFIRE R/ST Panel Mini and SPOTFIRE System

Analyte Isolate (Source ID)	Conc. 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%]
Streptococcus pyogenes	**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%]**

Analyte Isolate (Source ID)	Conc. 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	
(group A Strep) (ATCC 12344)	1.4E+03 cells/mL (3× 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			209/210 99.5%	210/210 100%	210/210 100%	**629/630 99.8%**	139/140 99.3%	137/140 97.9%	140/140 100%	**416/420 99.0%**	**1045/1050 99.5%** [98.9-99.9%]
Overall positive agreement (%) [95% Confidence Interval]											

3. Operator Questionnaire

Prior to the start of the Clinical Study, 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. Overall, the operators reported that the BIOFIRE SPOTFIRE R/ST 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. Since the SPOTFIRE R/ST Panel Mini only reports a sub-set of the larger panel organisms, and there is no change to formulation, sample types, or procedural steps of the device, these studies were deemed adequate to support the R/ST Panel Mini waiver submission. See K232954 for additional details.

M. Labeling for Waived Devices

The labeling consists of:

1. BIOFIRE SPOTFIRE R/ST Panel Mini Instructions For Use
2. BIOFIRE SPOTFIRE R/ST Panel Mini Quick Guide
3. BIOFIRE SPOTFIRE System Operator's Manual
4. BIOFIRE SPOTFIRE System Setup Quick Guide
5. BIOFIRE SPOTFIRE R/ST Panel and component labels

The following elements are appropriately present:

- The BIOFIRE SPOTFIRE System Operator's Manual specifies the environmental operating conditions under which testing may be performed.

- The BIOFIRE SPOTFIRE R/ST Panel Mini Quick Guide and BIOFIRE SPOTFIRE System Setup Quick Guide are clear and easy to understand.
- The BIOFIRE SPOTFIRE R/ST Panel Mini Instructions For Use and Quick Guide identify the test as CLIA Waived.
- The BIOFIRE SPOTFIRE R/ST 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 or uncertain 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.