510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K213954

B Applicant

BIOFIRE Diagnostics

C Proprietary and Established Names

BIOFIRE SPOTFIRE Respiratory Panel

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QOF	Class II	21 CFR 866.3981 - Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The Sars-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test	MI - Microbiology
OEM	Class II	21 CFR 866.3980 - Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
OOU	Class II	21 CFR 866.3980 - Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
OTG	Class II	21 CFR 866.3980 - Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
OZE	Class II	21 CFR 866.3980 - Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
OZX	Class II	21 CFR 866.3980 - Respiratory viral panel	MI - Microbiology

Product Code(s)	Classification	Regulation Section	Panel
		multiplex nucleic acid assay	
OZY	Class II	21 CFR 866.3980 - Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
OZZ	Class II	21 CFR 866.3980 - Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
OCC	Class II	21 CFR 866.3980 - Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
NSU	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH – Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the BIOFIRE SPOTFIRE Respiratory (R) Panel performed on the BIOFIRE SPOTFIRE System.

B Measurand:

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

C Type of Test:

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

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

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

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

Viruses:

Adenovirus

Coronavirus (seasonal)

Coronavirus SARS-CoV-2

Human Metapneumovirus

Human Rhinovirus/Enterovirus

Influenza A Virus

Influenza A virus A/H1-2009

Influenza A virus A/H3

Influenza B Virus

Parainfluenza Virus

Respiratory Syncytial Virus

Bacteria:

Bordetella parapertussis

Bordetella pertussis

Chlamydia pneumoniae

Mycoplasma pneumoniae

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

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

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

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:

BIOFIRE SPOTFIRE System

IV Device/System Characteristics:

A Device Description:
The BIOFIRE SPOTFIRE Respiratory (R) Panel (SPOTFIRE R Panel) is a multiplexed nucleic acid-amplification-based test that is intended to detect and identify 13 different respiratory viral and bacterial pathogens in nasopharyngeal swab (NPS) specimens from individuals with signs and symptoms of respiratory tract infection. The SPOTFIRE R Panel is performed on 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 closed system. The resulting PCR products are evaluated using assay-specific DNA melting analysis. The BIOFIRE SPOTFIRE System Software executes the SPOTFIRE R Panel test and interprets and reports the test results in approximately 15 minutes without user intervention.

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 up to three 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 Reagent Kit includes the following components:

- BIOFIRE SPOTFIRE R Panel Pouches
    - Each packed under vacuum in a metal canister and outer bag
- BIOFIRE Sample Preparation Reagent Kit (SPRK)
    - A fixed volume transfer pipette for addition of the test sample to the Sample Injection Vial
    - BIOFIRE Sample Buffer ampoule for addition to the Sample Injection Vial
    - BIOFIRE Sample Injection Vial for mixing of the test sample and Sample Buffer
    - BIOFIRE Hydration Injection Vial containing Hydration Solution for pouch rehydration

B Principle of Operation:

The BIOFIRE SPOTFIRE Panel Pouch is a closed-system disposable that contains all the reagents needed for sample preparation, reverse transcription, PCR amplification and fluorescent detection, including melt curve analysis of amplified products. The rigid plastic "fitment" of the pouch contains reagents in freeze-dried form while the flexible plastic portion of the pouch is divided into discrete "blisters" in which different chemical processes are carried out. After sample collection, the user injects hydration solution and the sample combined with BIOFIRE Sample Buffer into the pouch, places the pouch into the SPOTFIRE System, and starts the run. All other operations are automated.

The SPOTFIRE System Software is designed to run on the SPOTFIRE Control Station which consists of an integrated computer and touchscreen. The SPOTFIRE Systems Software interfaces with the user via the touchscreen to operate the instrument, collect, save, analyze and retrieve data, report test results and view stored information. Meanwhile the BIOFIRE SPOTFIRE R Panel Software works with the System Software and specifies the assay-specific parameters to perform the test, interpret the data and display the reported results.

To perform a test with SPOTFIRE R Panel, the sample is first mixed with a denaturing Sample Buffer to inactivate nucleases. The user then loads the diluted sample into the SPOTFIRE R Panel Pouch via the injection port. The vacuum in the pouch draws the liquid into the sample well of the pouch fitment and in the process rehydrates the cell-based Process Control. The sample mixture is moved to the lysis blister where mechanical agitation with zirconia-silica beads is used to complete cell lysis. The lysate is then mixed with glass-coated magnetic beads which adsorb the nucleic acids liberated in the lysis step. The extracted nucleic acids are washed to remove potential inhibitors and then eluted into buffer that is used to rehydrate the contents of the first stage PCR blister, which includes all the components needed for reverse transcription (RT) and first stage PCR. RT-PCR is initiated upon achievement of the requisite temperature within the amplification chamber. Following completion of first-stage amplification, the products of the reaction are diluted and combined with fresh reagents that are distributed over a second stage PCR array. The second stage primers are internal ("nested") with respect to the first stage primers and each analyte or control target is represented by three discrete amplification reactions. The second stage PCR array includes an assay control, the PCR2 Control, failure of which indicates a failure in the second stage PCR and invalidates the run.

Included in the PCR master mix for the second stage PCR is a fluorescent dye (LC Green Plus) that is specific for double-stranded DNA. The dye intercalates with the double stranded-PCR products and fluoresces at a specific wavelength that is detected by the SPOTFIRE System. At the end of the second stage PCR, the temperature of the array is gradually raised at a controlled ramp rate and the fluorescence is monitored. Fluorescence decreases at the melting temperature of the amplicons, and the temperature at which this occurs is characteristic of each amplification product. The software automatically evaluates the calculated melting temperature (Tm) in comparison to the predefined range for each specific analyte. The results from each of the three amplification reactions for each analyte are combined by the software to determine the test result. For an assay to be called positive, at least two of the three wells must have a PCR product with a Tm that is within the specified range for that analyte. Assays that do not meet these criteria are reported as negative.

Positive and negative results for the assay controls (Process Control and PCR2 Control) are compared to their expected values and assigned a "pass" or "fail" result. Panel-specific rules define how control failures affect interpretation of test results. For the SPOTFIRE R Panel, failure of either the RNA Process Control or PCR2 Control invalidates the results for all analytes. The final sample result is determined by the interpretation rules defined in the panel software.

For most bacteria and viruses detected by the SPOTFIRE R Panel, a "positive" result is reported if two or more of the wells corresponding to a single assay contain detectable PCR product of the appropriate Tm. However, to ensure inclusivity, certain analytes are targeted by multiple assays, the results of which are interpreted in combination, as described below in **Tables 1** and **2**.

Table 1. Interpretation of results for analytes targeted by multiple assays

Analyte	Number of Independent Assays	Positive Result Interpretation
Adenovirus	4	≥ 1 assay positive
Coronavirus (seasonal)	4	≥ 1 assay positive
Influenza A	4	≥ 2 assays positive [1]
Parainfluenza Virus	4	≥ 1 assay positive
SARS-CoV-2	2	≥ 1 assay positive

[1] At least one of the pan-influenza A assays and *either* the H1-2009- *or* the H3-specific assay must produce positive results to report a positive subtype result (**Table 2**)

Table 2. Possible result permutations for influenza A from patient samples

Assay				Result Reported	Action Required
FluA-pan1	FluA-pan2	FluA-H1-2009	FluA-H3		
Positive	Positive	Positive	Negative	POSITIVE Influenza A Virus Subtype H1-2009	Report
Positive	Negative	Positive	Negative		
Negative	Positive	Positive	Negative		
Positive	Positive	Negative	Positive	POSITIVE Influenza A Virus Subtype H3	Report
Positive	Negative	Negative	Positive		
Negative	Positive	Negative	Positive		
Positive	Positive	Positive	Positive	POSITIVE Influenza A Virus Multiple Subtypes	Report
Positive	Negative	Positive	Positive		
Negative	Positive	Positive	Positive		
Positive	Positive	Negative	Negative	POSITIVE Influenza A Virus No Subtype	Re-test [1]
Positive	Negative	Negative	Negative	UNCERTAIN Influenza A Virus	
Negative	Positive	Negative	Negative		
Negative	Negative	Positive	Negative		
Negative	Negative	Negative	Positive		
Negative	Negative	Positive	Positive		
Negative	Negative	Negative	Negative	NEGATIVE	Report

[1] If the re-test result is the same, the user is instructed to contact public health authorities for confirmatory testing

C Instrument Description Information:

1. Instrument Name:

   BIOFIRE SPOTFIRE System

2. Specimen Identification:

   Specimen identification can be entered manually or by scanning a barcode.

3. Specimen Sampling and Handling:

Use of the SPOTFIRE R Panel requires a nasopharyngeal swab (NPS) to be collected according to standard procedures and placed in 1-3 mL of viral 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 (15-25 °C) or for up to 3 days at 2-8 °C or up to 30 days at ≤ -15 °C.

The SPOTFIRE System Software includes step-by-step on-screen instructions that guide the user through the process of starting a run on the instrument. After cleaning the work area and Pouch Loading Station, the user removes a SPOTFIRE R Panel pouch from its vacuum packaging and places it into the Pouch Loading Station. They then hydrate the pouch using the Hydration Injection Vial by injecting the contents through the Hydration Solution Injection Port, after which they transfer a fixed volume of sample to the Sample Injection Vial, together with the entire contents of the Sample Buffer ampoule. After mixing the Sample Injection Vial by invertion, 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, including interpretation of results.

4. Calibration:

The BIOFIRE SPOTFIRE System is factory calibrated. User calibration is not required.

5. Quality Control:

Internal Controls
Two process controls are included in each SPOTFIRE R Panel pouch:

1) *RNA Process Control*
The RNA Process Control comprises freeze-dried *Schizosaccharomyces pombe* which is rehydrated when the sample is loaded. The control material is carried through all stages of the test process, including nucleic acid extraction and purification, reverse transcription, first and second stage PCR amplification and DNA melt analysis. A positive result indicates that all process steps carried out in the SPOTFIRE R Panel pouch were completed successfully.

2) *PCR2 Control*
The PCR2 control comprises a DNA target sequence that is dried into specific wells used for the second stage amplification and melting curve analysis. A positive result indicates that these processes were successful.

A positive result is required for both the RNA Process Control and the PCR2 Control for the results for an individual pouch to be considered valid. If either control fails, the sample should be retested using a new pouch.

External Controls
BIOFIRE recommends testing External Positive and Negative Controls that are commercially available from Maine Molecular Quality Controls, Inc. when training a new operator, or when a new shipment of SPOTFIRE panels or a new control station is received:

- SPOTFIRE RSP Positive Control (M42638)
- SPOTFIRE RSP Negative Control (M42738)

These controls are designed to monitor the performance of each of the individual assays within the SPOTFIRE R Panel and should be tested in accordance with their FDA-cleared Instructions For Use (K221253).

The Instructions For Use of the SPOTFIRE R Panel indicate that an External Negative Control should be tested at least monthly to monitor for environmental contamination. Additional External Controls may be tested as needed.

External Positive and Negative Controls were tested during the Prospective Clinical Study described in **Section VII (C)**. In total, 252 External Controls were tested of which 8 were associated with instrument errors, 4 were tested using an incorrect version of the reagent pouch and 4 were tested using the workflow for patient samples rather than Quality Control. As a result, there were 236 External Controls with valid results (109 Positive and 131 Negative). Of these, 99.1% of the External Positive Controls (108/109) and 100% of the External Negative Controls (131/131) produced the expected results.

V Substantial Equivalence Information:

A Predicate Device Name(s):
BioFire Respiratory Panel 2.1 (RP2.1)

B Predicate 510(k) Number(s):
DEN200031

C Comparison with Predicate(s):

Device & Predicate Device(s):	K213954	DEN200031
Device Trade Name	BIOFIRE SPOTFIRE Respiratory (R) Panel	BIOFIRE Respiratory Panel 2.1
General Device Characteristic Similarities		
Intended Use/Indications For Use	The BIOFIRE SPOTFIRE Respiratory (R) Panel (SPOTFIRE R Panel) is a multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE SPOTFIRE System for the simultaneous, qualitative	The BIOFIRE Respiratory Panel 2.1 (RP2.1) is a PCR-based multiplexed nucleic acid test intended for use with the BIOFIRE FilmArray 2.0 or BIOFIRE FilmArray Torch systems for the simultaneous qualitative detection and

Device & Predicate Device(s):	K213954	DEN200031
	detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab (NPS) specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19. The following organism types and subtypes are identified and differentiated using the SPOTFIRE R Panel: *Viruses:* • Adenovirus • Coronavirus (seasonal) • Coronavirus SARS-CoV-2 • Human metapneumovirus • Human rhinovirus/enterovirus • Influenza A virus ○ Influenza A virus A/H1-2009 ○ Influenza A virus A/H3 • Influenza B virus • Parainfluenza virus • Respiratory syncytial virus *Bacteria:* • *Bordetella parapertussis* • *Bordetella pertussis* • *Chlamydia pneumoniae* • *Mycoplasma pneumoniae* Nucleic acids from the viral and bacterial organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification	identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections, including COVID-19. The following organism types and subtypes are identified using the BIOFIRE RP2.1: • Adenovirus, • Coronavirus 229E • Coronavirus HKU1 • Coronavirus NL63 • Coronavirus OC43 • Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) • Human Metapneumovirus • Human Rhinovirus/Enterovirus • Influenza A, including subtypes H1, H1-2009, and H3 • Influenza B • Parainfluenza Virus 1 • Parainfluenza Virus 2 • Parainfluenza Virus 3 • Parainfluenza Virus 4 • Respiratory Syncytial Virus • *Bordetella parapertussis* (IS1001) • *Bordetella pertussis* (ptxP) • *Chlamydia pneumoniae*, and • *Mycoplasma pneumoniae* Nucleic acids from the respiratory viral and

Device & Predicate Device(s):	K213954	DEN200031
	of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection are indicative of the presence of the identified microorganism and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the SPOTFIRE R Panel may not be the definite cause of disease. Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.	bacterial organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection is indicative of the presence of the identified microorganism and aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the BIOFIRE RP2.1 may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating

Device & Predicate Device(s):	K213954	DEN200031
		a patient with possible respiratory tract infection.
Specimen Types	Same	Nasopharyngeal swabs
Patient Population	Individuals with signs and symptoms of respiratory tract infection	Individuals suspected of respiratory tract infections, including COVID-19
Organisms Detected	Same except: Seasonal Coronaviruses are not differentiated Parainfluenza viruses are not differentiated No subtyping assay for Influenza Virus A/H1 (pre-pdm2009)	*Viruses:* Adenovirus Coronavirus 229E Coronavirus HKU1 Coronavirus NL63 Coronavirus OC43 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Human Metapneumovirus Human Rhinovirus/Enterovirus Influenza A Subtypes: H1, H3, H1-2009 Influenza B Parainfluenza Virus 1 Parainfluenza Virus 2 Parainfluenza Virus 3 Parainfluenza Virus 4 Respiratory Syncytial Virus *Bacteria:* *Bordetella parapertussis* *Bordetella pertussis* *Chlamydia pneumoniae* *Mycoplasma pneumoniae*
Technology	Same	Highly multiplexed PCR with DNA melting analysis
General Device Characteristic Differences		
Instrument	BIOFIRE SPOTFIRE System	BIOFIRE FilmArray 2.0 or BIOFIRE FilmArray Torch Systems
Disease Syndrome	Same	Respiratory tract infection
Time to Result	~15 minutes	~45 minutes

VI Standards/Guidance Documents Referenced:

- Guidance for Clinical Laboratories, Commercial Manufactures, and FDA Staff – Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (2020)
- Guidance for Industry - Part 11, Electronic Records; Electronic Signatures - Scope and Application (August 2003)
- Guidance for Industry - Computerized Systems Used in Clinical Investigations (May 2007)
- Guidance for Industry - Oversight of Clinical Investigations - A Risk-Based Approach to Monitoring (August 2013)
- Guidance for Industry - Electronic Source Data in Clinical Investigations (September 2013)
- Guidance for Industry and FDA Staff - Acceptance of Clinical Data to Support Medical Device Applications and Submissions - Frequently Asked Questions (February 2018)
- Guidance for Industry and FDA Staff - Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of *In Vitro* Diagnostic Devices (February 2020)
- Guidance for Industry and FDA Staff - Recommendations for Dual 510(k) and CLIA Waiver by Application Studies (February 2020)
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay (October 2009)
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Testing for Detection and Differentiation of Influenza A Virus Subtypes Using Multiplex Nucleic Acid Assays (October 2009)
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Testing for Human Metapneumovirus (hMPV) Using Nucleic Acid Assays (October 2009)
- Guidance for Industry and FDA Staff - Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests, (March 2007)
- Guidance for Industry and FDA Staff, Evaluation and Reporting of Age-, Race-, and Ethnicity-Specific Data in Medical Device Clinical Studies (September 2017)
- Guidance for Sponsors, Investigators, and IRBs - Impact of Certain Provisions of the Revised Common Rule on FDA-Regulated Clinical Investigations (October 2018)
- Guidance for Sponsors, Institutional Review Boards, C and FDA Staff - Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable (April 2006)
- Guidance for Industry and Food and Drug Administration Staff - Highly Multiplexed Microbiological/Medical Countermeasure *In Vitro* Nucleic Acid Based Diagnostic Devices (August 2014)
- Guidance for Industry and FDA Staff - Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests (March 2007)
- Guidance for Industry and FDA Staff - Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices (May 2005)
- Guidance for Industry and FDA Staff - Off-The-Shelf Software Use in Medical Devices (September 2019)
- Final Guidance for Industry and FDA Staff - General Principles of Software Validation (January 2002)
- Guidance for Industry and FDA Staff - Content of Premarket Submissions for

Management of Cybersecurity in Medical Devices (October 2014)

- CLSI. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition. CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
- CLSI. Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline. CLSI document EP25-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
- CLSI. Interference Testing in Clinical Chemistry. 3rd ed. CLSI guideline EP07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
- ICH Harmonised Guideline: Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2) (November 2016)
- GHTF: Clinical Evidence for IVD Medical Devices - Clinical Performance Studies for *In Vitro* Diagnostic Medical Devices (November 2012)
- WMA Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects
- ISO 14971:2019 Medical devices - Application of risk management to medical devices
- IEC 62366-1:2015 Medical devices - Part 1: Application of usability engineering to medical devices
- ISO 62304:2006 Medical device software - Software life-cycle processes
- ISO 15223-1:2016: Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied - Part 1: General requirements
- IEC 61010-1 Ed. 3.0 2010: Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements
- EN 61010-2-101:2002/IEC 61010-2-101:2015: Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101: Particular requirements for *in vitro* diagnostic (IVD) medical equipment.
- IEC 60601-1-2:2014 (Edition 4.0): Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance - Collateral Standard: Electromagnetic disturbances - Requirements and tests
- AIM 7351731 Rev 2.00 (2017-02-23): Medical Electrical Equipment and System Electromagnetic Immunity Test for Exposure to Radio Frequency Identification Readers
- UL 2900-1 Ed. 1 2017: Standard for Software Cybersecurity Network-Connectable Products, Part I: General Requirements
- ANSI UL 2900-2-1: Standard for Safety, Software Cybersecurity for Network-Connectable Products, Part 2-1: Particular Requirements for Network Connectable Components of Healthcare and Wellness Systems
- ISO 13485:2016/EN ISO 13485:2016; Medical devices - Quality Management System - Requirements for regulatory purposes
- ISO 20916:2019; *In vitro* diagnostic medical devices. Clinical performance studies using specimens from human subjects. Good study practice
- EN 13612:2002; Performance evaluation of *in vitro* diagnostic medical devices
- EN ISO 18113-1:2011; *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labeling). Terms, definition and general requirements
- EN ISO 18113-2:2011; *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labeling) – Part 2: *In vitro* diagnostic reagents for professional use
- EN ISO 18113-3:2011; *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labeling) – Part 3: *In vitro* diagnostic instruments for professional use
- EN ISO 23640:2015; *In vitro* diagnostic medical devices - Evaluation of stability of *in vitro*

diagnostic reagents
- EN 13641:2002; Elimination or reduction of risk of infection related to *in vitro* diagnostics reagents
- IEC 61326-1:2012; Electrical equipment for measurement control and laboratory use - EMC requirements - Part 1: General requirements
- EN 61326-2-6:2006/IEC 61326-2-6:2012; Electrical equipment for measurement control and laboratory use - EMC requirements - Part 2-6: Particular requirements - *In vitro* diagnostic (IVD) medical equipment

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

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

Table 3. Contrived samples used to evaluate the precision/reproducibility of the SPOTFIRE R Panel

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

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

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

At the external study sites, testing was performed with a single SPOTFIRE System at each site and occurred over five non-consecutive days using a single lot of reagents that was common across each of the sites. On each day, two operators each tested two replicates of each sample for a total of 60 replicates per sample overall (2 operators x 2 samples x 3 sites x 5 days = 60 replicates).

For each analyte there were 150 positive and 600 negative samples. Samples that produced an invalid result on initial testing due to process control failure (n = 12) or instrument error (n = 2) were retested. Precision and reproducibility for each analyte were calculated by assessing the agreement between the reported test results and the expected results for each sample.

A summary of the results of the study, stratified by analyte and SPOTFIRE system/site is provided in **Table 4**. Positive agreement for all analytes was > 95% and there was negligible difference between instruments, sites, reagent lots or operators. Negative agreement was 100% in all cases.

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

Table 4. Summary of results from the Precision and Reproducibility Study, stratified by SPOTFIRE instrument and study site

Assay/Analyte		Expected Result	Agreement (%) Stratified by Site and SPOTFIRE System						Total
			BIOFIRE Diagnostics			External Site			
			A	B	C	1	2	3	
Adenovirus		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
Adenovirus Species B Type 3		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Bordetella parapertussis		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
B. parapertussis E595		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Bordetella pertussis		Positive	29/30 (96.7)	29/30 (96.7)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	148/150 (98.7)
			88/90 (97.8)			60/60 (100)			
B. pertussis A639		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Chlamydia pneumoniae		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
Chlamydophila pneumoniae AR-39		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Seasonal Coronavirus	Coronovirus 229E	Positive	30/30 (100)	30/30 (100)	29/30 (96.7)	20/20 (100)	20/20 (100)	20/20 (100)	149/150 (99.3)
			89/90 (98.9)			60/60 (100)			
	Coronavirus OC43	Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
	Coronavirus NL63	Positive	27/30 (90.0)	30/30 (100)	30/30 (100)	18/20 (90.0)	20/20 (100)	18/20 (90.0)	143/150 (95.3)
			87/90 (96.7)			56/60 (93.3)			
	None	Negative	60/60 (100)	60/60 (100)	60/60 (100)	40/40 (100)	40/40 (100)	40/40 (100)	300/300 (100)
			180/180 (100)			120/120 (100)			

Assay/Analyte		Expected Result	Agreement (%) Stratified by Site and SPOTFIRE System						Total
			BIOFIRE Diagnostics			External Site			
			A	B	C	1	2	3	
Coronavirus SARS-CoV-2 SARS-CoV-2 2019-nCoV/USA-WA1/2020		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Human Metapneumovirus hMPV 3 Type B1		Positive	30/30 (100)	30/30 (100)	30/30 (100)	19/20 (95.0)	20/20 (100)	19/20 (95.0)	148/150 (98.7)
			90/90 (100)			58/60 (96.7)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Human Rhinovirus/ Enterovirus Enterovirus D68 US/MO/14-18947		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Influenza A Virus	Influenza A H1N1pdm	Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
	Influenza A H3N2	Positive	30/30 (100)	30/30 (100)	30/30 (100)	19/20 [3] (95.0)	19/20 (95.0)	20/20 (100)	148/150 (98.7)
			90/90 (100)			58/60 (96.7)			
	None	Negative	90/90 (100)	90/90 (100)	90/90 (100)	60/60 (100)	60/60 (100)	60/60 (100)	450/450 (100)
			270/270 (100)			180/180 (100)			
Influenza A Virus Subtype H1-2009 [1] Influenza A H1N1pdm Michigan/45/15		Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Influenza A Virus Subtype H3 [2] Influenza A H3N2 Hong Kong/4801/14		Positive	30/30 (100)	30/30 (100)	30/30 (100)	19/20 [3] (95.0)	19/20 (95.0)	20/20 (100)	148/150 (98.7)
			90/90 (100)			58/60 (96.7)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Influenza B Virus Influenza B B/Florida/02/06		Positive	29/30 (96.7)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	149/150 (99.3)
			89/90 (98.9)			60/60 (100)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Mycoplasma pneumoniae *M. pneumoniae* M129		Positive	29/30 (96.7)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	149/150 (99.3)
			89/90 (98.9)			60/60 (100)			
		Negative	120/120 (100)	120/120 (100)	120/120 (100)	80/80 (100)	80/80 (100)	80/80 (100)	600/600 (100)
			360/360 (100)			240/240 (100)			
Parainfluenza Virus	Parainfluenza Virus 1	Positive	30/30 (100)	30/30 (100)	30/30 (100)	20/20 (100)	20/20 (100)	20/20 (100)	150/150 (100)
			90/90 (100)			60/60 (100)			
	Parainfluenza Virus 2	Positive	30/30 (100)	29/30 (96.7)	28/30 (93.3)	20/20 (100)	20/20 (100)	20/20 (100)	147/150 (98.0)
			87/90 (96.7)			60/60 (100)			

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

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

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

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

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

The analytical specificity (exclusivity) of the SPOTFIRE R Panel was evaluated through a combination of *in silico* analysis and laboratory testing of on- and off-panel species, phylogenetically related viruses and bacteria and other microorganisms that may be found in the respiratory tract.

In silico Analysis of Specificity (Exclusivity)
In silico analysis of assay specificity/exclusivity was performed by conducting searches with whole amplicon and paired inner primer sequences using the NCBI BLAST "blastn" tool. The location and orientation of regions of homology with the primer sequences were evaluated to determine the potential for amplification. A summary of the results from the analysis is provided in **Table 5**. The SPOTFIRE R Panel assays were shown to be specific for their respective analytes with the following exceptions, which are noted in the device labeling:

- Cross-reaction of the SARS-CoV-2 assays with closely related bat and pangolin coronavirus sequences
- Cross-reaction of the Human Rhinovirus/Enterovirus assay with *B. pertussis*, *B. parapertussis* and *B. bronchiseptica*, in addition to bovine and canine picornaviruses

- Cross-reaction of the *B. parapertussis* assay with strains of *B. bronchiseptica* that carry IS1001
- Cross-reaction of the *C. pneumoni*ae assay with *Chlamydia gallinacea*

Table 5. Summary of *in silico* BLAST analysis to evaluate exclusivity

Assay	Predicted Cross-reaction
Adenovirus	None
	None
	None
	None
Seasonal Coronavirus	None
	None
	None
	None
SARS-CoV-2	Bat coronavirus Pangolin coronavirus Bat SARS-like coronavirus
Human Metapneumovirus	None
Human Rhinovirus/Enterovirus	Bovine and canine picornavirus *B. pertussis*, *B. parapertussis* and *B. bronchiseptica*
Influenza A Virus A/H1-2009 [1]	Swine H1 sequences
Influenza B Virus	None
Parainfluenza Virus	None
Respiratory Syncytial Virus	None
Bordetella parapertussis	Strains of *B. bronchiseptica* carrying IS1001
Bordetella pertussis	None
Chlamydia pneumoniae	*Chlamydia gallinacea*
Mycoplasma pneumoniae	None

[1] Certain avian H1N2 sequences (AY233393 and AY038014) may also be detected and reported as H1-2009
Note: A sequence from *Protopolystoma xenopodis* (a parasitic worm found in frogs) exhibited significant homology to the pan-influenza A PCR primers and may be amplified but this organism is of no clinical relevance

Laboratory Testing of Analytical Specificity
The analytical specificity (exclusivity) of the SPOTFIRE R Panel was also evaluated by testing high concentrations of on- and off-panel viruses, bacteria and other microorganisms that are phylogenetically related or may be found in nasopharyngeal and throat swab specimens. Analysis of on-panel analytes was performed to evaluate the potential for intra-panel cross-reactivity (i.e., detection of an analyte by an assay other than the target-specific assay for that analyte). Analysis of off-panel analytes was performed to evaluate the potential for cross-reaction with other respiratory flora that are not detected by the panel.
Each on-panel species was tested in triplicate and the results are shown in **Tables 6** and **7**. The expected results were obtained with each on-panel virus and no cross-reaction was observed (**Table 6**). Based on *in silico* analysis, it was predicted that *B. parapertussis* and *B. pertussis* would produce positive results with the Human Rhinovirus/Enterovirus assay. *B. pertussis* produced false-positive results at 1.3×10^{10} CFU/mL but was reported negative when tested at 1.3×10^{9} CFU/mL (**Table 7**). False-positive results were not observed with *B.*

parapertussis at the concentration tested (4.6 x 10^9 CFU/mL). The potential for cross-reaction of the human Rhinovirus/Enterovirus assay with both *B. parapertussis* and *B. pertussis* is noted in the device labeling.

Table 6. Results from cross-reactivity testing with "on-panel" viruses

Species	Source Identity	Concentration (per mL)	Observed Cross-reaction
Adenovirus A	Zeptometrix 0810073CF	1.4 x 10^5 $TCID_{50}$	None
Adenovirus B	Zeptometrix 0810062CF	1.2 x 10^7 $TCID_{50}$	None
Adenovirus C	Zeptometrix 0810110CF	2.2 x 10^6 $TCID_{50}$	None
Adenovirus D	Zeptometrix 0810119CF	1.7 x 10^5 $TCID_{50}$	None
Adenovirus E	Zeptometrix 081070CF	1.5 x 10^5 $TCID_{50}$	None
Adenovirus F	Zeptometrix 0810085CF	1.1 x 10^6 $TCID_{50}$	None
Coronavirus 229E	ATCC VR-740	8.9 x 10^6 $TCID_{50}$	None
Coronavirus HKU1	Clinical specimen-1 [1]	4.5 x 10^7 copies	None
	Clinical specimen-2 [2]		None [3]
Coronavirus NL63	Zeptometrix 0810228CF	5.0 x 10^5 $TCID_{50}$	None
Coronavirus OC43	Zeptometrix 0810024CF	3.6 x 10^5 $TCID_{50}$	None
Coronavirus SARS-CoV-2	ATCC VR-1986HK	7.6 x 10^7 copies	None
Enterovirus D68	ATCC VR-1823	1.6 x 10^7 $TCID_{50}$	None
Human Metapneumovirus A1	Zeptometrix 0810161CF	2.5 x 10^5 $TCID_{50}$	None
Human Metapneumovirus A2	Zeptometrix 0810164CF	3.6 x 10^5 $TCID_{50}$	None
Human Metapneumovirus B1	Zeptometrix 0810156CF	1.6 x 10^4 $TCID_{50}$	None
Human Metapneumovirus B2	Zeptometrix 0810162CF	1.3 x 10^6 $TCID_{50}$	None
Rhinovirus A1	Zeptometrix 0810012CFN	1.3 x 10^6 $TCID_{50}$	None
Influenza A H1N1pdm09	Zeptometrix 08100538CF	1.4 x 10^5 $TCID_{50}$	None
Influenza A H3N2	Zeptometrix 010526CF	7.2 x 10^5 $TCID_{50}$	None
Influenza B (Victoria)	BEI NR-44023	2.8 x 10^8 $CEID_{50}$	None
	Zeptometrix 0810037CF	2.5 x 10^5 $TCID_{50}$	None
Influenza B (Yamagata)	Zeptometrix 0810256CF	2.1 x 10^4 $TCID_{50}$	None
Parainfluenza Virus 1	Zeptometrix 0810014CF	4.2 x 10^5 $TCID_{50}$	None
Parainfluenza Virus 2	Zeptometrix 0810015CF	1.2 x 10^7 $TCID_{50}$	None
Parainfluenza Virus 3	Zeptometrix 0810016CF	3.4 x 10^7 $TCID_{50}$	None
Parainfluenza Virus 4a	Zeptometrix 0810060CF	3.4 x 10^7 $TCID_{50}$	None
Respiratory Syncytial Virus A	Zeptometrix 0810040ACF	4.2 x 10^5 $TCID_{50}$	None
Respiratory Syncytial Virus B	Zeptometrix 0810479CF	4.2 x 10^5 $TCID_{50}$	None

$CEID_{50}$: Chicken Egg Infectious Dose-50%; $TCID_{50}$: Tissue Culture Infectious Dose-50%

[1] Previously reported positive for Parainfluenza Virus 4 in addition to Coronavirus HKU1

[2] Previously reported positive for Adenovirus in addition to Coronavirus HKU1

[3] 1/3 replicates positive for Adenovirus (not considered cross-reactivity based on previous test results for the source specimen)

Table 7. Results from cross-reactivity testing with "on-panel" bacteria

Species	Source Identity	Concentration (per mL)	Observed Cross-reaction
Bordetella parapertussis	Zeptometrix 0801462	4.6 x 10^9 CFU	None [1]
Bordetella pertussis	Zeptometrix 0801459	1.3 x 10^{10} CFU	Human Rhinovirus/ Enterovirus [2]
		1.3 x 10^9 CFU	None
Chlamydia pneumoniae	ATCC 53592	2.9 x 10^7 IFU	None
Mycoplasma pneumoniae	Zeptometrix 0801579	2.5 x 10^7 CCU	None

CCU: Color Changing Units; CFU: Colony Forming Units; IFU: Inclusion Forming Units

[1] *In* silico analysis predicted detection by the Human Rhinovirus/Enterovirus assay but none was observed

[2] Cross-reaction also predicted by *in silico* analysis

The potential for cross-reaction with off-panel analytes was evaluated by testing 87 stains of of bacteria, 2 species of fungus and 7 viruses that are either phylogenetically related to the on-panel analytes or which are commonly found in the respiratory tract (**Table 8**). No cross-reaction was observed other than the detection of one strain of *B. bronchiseptica* by the assay for *B. parapertussis* that was predicted by *in silico* analysis.

Table 8. Results from cross-reactivity testing with "off-panel" bacteria, viruses and fungi

Species	Source Identity	Concentration (per mL)	Observed Cross-reaction
Bacteria			
Arcanobacterium bernardiae	ATCC BAA-441	1.6 x 10^9 cells	None
Arcanobacterium haemolyticum	ATCC 9345	1.5 x 10^8 cells	None
Arcanobacterium pyogenes	ATCC 49698	6.7 x 10^9 cells	None
Bacillus cereus	ATCC 7064	8.3 x 10^9 cells	None
Bordetella bronchiseptica	ATCC 10580	8.3 x 10^9 cells	None
	ATCC 4617	7.9 x 10^9 cells	None
	ATCC 19395	7.9 x 10^9 cells	None
	NRRL B-59914	7.1 x 10^9 cells	None
	NRRL B-59909	28 cells	*B. parapertussis* [1]
		2.8 cells	*B. parapertussis* [1]
Bordetella holmesii	ATCC 700052	8.3 x 10^9 cells	None
Burholderia cepacia	ATCC 51671	7.9 x 10^9 cells	None
Campylobacter rectus	ATCC 33238	7.6 x 10^7 cells	None
Chlamydia trachomatis	Zeptometrix 0801775	1.3 x 10^8 IFU	None
Corynebacterium diphtheriae	ATCC 27010	8.0 x 10^9 cells	None
Corynebacterium pseudodiphtheriticum	ATCC 10700	8.7 x 10^9 cells	None
Enterococcus casseliflavus	ATCC 49605	8.0 x 10^9 cells	None
Enterococcus faecalis	Zeptometrix 0801637	8.0 x 10^9 cells	None
Escherichia coli	ATCC BAA-2196	7.2 x 10^9 cells	None
Fusobacterium nucleatum	ATCC 25586	4.9 x 10^8 cells	None
Fuobacterium necrophorum subsp. *funduliforme*	ATCC 51357	4.4 x 10^8 cells	None
Fusobacterium varium	ATCC 27725	1.6 x 10^8 cells	None
Gemella haemolysans	ATCC 10379	4.0 x 10^9 cells	None
Gemella morbillorum	ATCC 27824	1.0 x 10^8 cells	None
Granulicatella adiacens	ATCC 49175	1.3 x 10^9 cells	None
Haemophilus influenzae	ATCC 10211	8.3 x 10^9 cells	None

Species	Source Identity	Concentration (per mL)	Observed Cross-reaction
Haemophilus parahaemolyticus	ATCC 49700	8.7 x 10^9 cells	None
Klebsiella pneumoniae	CDC AR-BANK#0115	7.3 x 10^9 cells	None
Lactobacillus rhamnosus	ATCC 7469	7.9 x 10^9 cells	None
Lactobacillus lactis	ATCC 29146	6.2 x 10^9 cells	None
Legionella pneumophila	ATCC 33215	7.0 x 10^9 cells	None
Leptotrichia buccalis	ATCC 14201	4.4 x 10^8 cells	None
Moraxella catarrhalis	ATCC 43627	7.2 x 10^9 cells	None
Mycobacterium tuberculosis	Zeptometrix 0801660	6.1 x 10^6 cells	None
Mycoplasma buccale	Mycoplasma Experience NC10136	1.4 x 10^7 CFU	None
Mycoplasma faucium	Mycoplasma Experience NC10174	1.4 x 10^6 CFU	None
Mycoplasma fermentans	Mycoplasma Experience NC10117	2.8 x 10^7 CFU	None
Mycoplasma genitalium	Mycoplasma Experience NC10195	1.8 x 10^6 CFU	None
Mycoplasma hominis	Mycoplasma Experience NC10111	1.2 x 10^7 CFU	None
Mycoplasma lipophilum	Mycoplasma Experience NC10173	1.5 x 10^6 CFU	None
Mycoplasma orale	Mycoplasma Experience NC10112	2.2 x 10^7 CFU	None
Mycoplasma salivarium	Mycoplasma Experience NC10113	4.4 x 10^6 CFU	None
Neisseria elongata	ATCC 25295	8.5 x 10^9 cells	None
Neisseria gonorrhoeae	Zeptometrix 0801482	4.9 x 10^7 CFU	None
Neisseria lactamica	ATCC 23971	2.7 x 10^9 cells	None
Neisseria meningitidis	ATCC 13113	7.4 x 10^9 cells	None
Neisseria sicca	ATCC 9913	7.2 x 10^9 cells	None
Neisseria subflava	ATCC 49275	8.0 x 10^9 cells	None
Parvimonas micra	ATCC 33270	6.0 x 10^7 cells	None
Pneumocystis carinii	ATCC PRA-159	1.0 x 10^7 nuclei	None
Porphyromonas endodontalis	ATCC 35406	1.6 x 10^7 cells	None
Porphyromonas gingivalis	ATCC BAA-308	5.0 x 10^8 cells	None
Prevotella histicola	BEI HM-471	9.0 x 10^8 cells	None
Prevotella melaninogenica	ATCC 25845	6.9 x 10^8 cells	None
Prevotella oralis	ATCC 33322	6.2 x 10^8 cells	None
Pseudomonas aeruginosa	CDC AR-BANK#0092	8.3 x 10^9 cells	None
Rhodococcus equi	ATCC 33706	7.3 x 10^9 cells	None
Serratia marcescens	ATCC 27137	8.9 x 10^9 cells	None
Staphylococcus aureus	ATCC BAA-1700	7.4 x 10^9 cells	None
Staphylococcus epidermidis	ATCC 12228	8.0 x 10^9 cells	None
Staphylococcus haemolyticus	ATCC 29968	8.0 x 10^9 cells	None
Staphylococcus intermedius	ATCC 29663	8.2 x 10^9 cells	None
Staphylococcus saprophyticus	ATCC 15305	8.1 x 10^9 cells	None
Streptococcus agalactiae	ATCC 13813	6.0 x 10^9 cells	None
Streptococcus anginosus	ATCC 700231	7.1 x 10^9 cells	None
Streptococcus constellatus subsp. *pharyngitis*	NCTC 13122	5.6 x 10^8 cells	None
Streptococcus dysgalactiae subsp. *dysgalactiae*	ATCC 43078	6.7 x 10^9 cells	None
	NCTC 4669	7.4 x 10^9 cells	None
	NCTC 4335	8.4 x 10^9 cells	None

Species	Source Identity	Concentration (per mL)	Observed Cross-reaction
	NCTC 4670	6.6 x 10^9 cells	None
	CCUG 27665	7.4 x 10^9 cells	None
	CCUG 28112	6.7 x 10^9 cells	None
	CCUG 28114	7.5 x 10^9 cells	None
Streptococcus dysgalactiae subsp. *equisimilis* (human)	Zeptometrix 0801516	7.8 x 10^8 CFU	None
Streptococcus dysgalactiae subsp. *equisimilis* (pig)	CCUG 28117	7.1 x 10^9 cells	None
Streptococcus dysgalactiae subsp. *equisimilis* (horse)	CCUG 27664	7.5 x 10^9 cells	None
	ATCC 10009	6.9 x 10^9 cells	None
Streptococcus gallolyticus	ATCC 43143	2.8 x 10^9 cells	None
Streptococcus gordonii	ATCC 10558	4.5 x 10^9 cells	None
Streptococcus intermedius	ATCC 27335	2.9 x 10^9 cells	None
Streptococcus mitis	ATCC 15914	3.2 x 10^9 cells	None
Streptococcus mutans	ATCC 25175	2.3 x 10^9 cells	None
Streptococcus oralis	ATCC 10557	1.1 x 10^9 cells	None
Streptococcus parasanguinis	ATCC 15912	7.8 x 10^9 cells	None
Streptococcus pneumoniae	ATCC 49619	2.5 x 10^8 cells	None
Streptococcus pyogenes	ATCC 12344	3.4 x 10^8 cells	None
Streptococcus salivarius	ATCC 13419	6.6 x 10^9 cells	None
Streptococcus sanguinis	ATCC 10556	1.1 x 10^9 cells	None
Tannerella forsythia	ATCC BAA-2717	2.6 x 10^8 cells	None
Treponema denticola	ATCC 33520	2.2 x 10^8 cells	None
Ureaplasma urealyticum	ATCC 27618	5.7 x 10^7 cells	None
Veillonella parvula	ATCC 10790	4.7 x 10^8 cells	None
Yeast			
Candida albicans	ATCC MYA-2876	2.8 x 10^8 cells	None
Saccharomyces cerevisiae	ATCC 18824	1.9 x 10^8 cells	None
Viruses			
Cytomegalovirus	Zeptometrix 0810003CF	1.9 x 10^5 $TCID_{50}$	None
Epstein Barr Virus	Zeptometrix 0810008CF	5.9 x 10^6 copies	None
Human Herpes Simplex Virus 1	ATCC VR-260	8.9 x 10^6 $TCID_{50}$	None
Measles Virus	Zeptometrix 0810025CF	2.5 x 10^5 $TCID_{50}$	None
Middle East Respiratory Syndrome Coronavirus	Zeptometrix 0810575CFHI	1.2 x 10^5 $TCID_{50}$	None
Mumps virus	Zeptometrix 0810079CF	2.0 x 10^5 $TCID_{50}$	None
Severe Acute Respiratory Syndrome Coronavirus	BEI NR-52346	5.3 x 10^5 genomes	None

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

[1] *B. bronchiseptica* NRRL B-59909 carries IS1001 which is the target for the SPOTFIRE R Panel *B. parapertussis* assay. Positive results were obtained with all target levels ≥ 28 cells/mL and with 1/3 replicates at 2.8 cells/mL.

Based on a combination of the *in silico* and laboratory testing data, the potential for cross-reaction of the SPOTFIRE R Panel with certain on- and off-panel analytes is noted in the device labeling, as summarized in **Table 9**.

Table 9. Summary of known or predicted cross-reactivity

SPOTFIRE R Panel Assay	Cross-reactive Species	Description
SARS-CoV-2	Bat coronavirus Pangolin coronavirus Bat SARS-like coronavirus	Predicted cross-reaction with closely related sarbecoviruses isolated from bats and pangolin
Bordetella parapertussis	*Bordetella bronchiseptica* (isolates carrying IS1001)	Some strains of *B. bronchiseptica* carry IS1001, the target for the *B. parapertussis* assay
Human Rhinovirus/Enterovirus	*Bordetella bronchiseptica* *Bordetella parapertussis* *Bordetella pertussis* Bovine and canine picornaviruses	The Human Rhinovirus/Enterovirus assay may amplify homologous sequences in the listed organisms
Influenza A Virus A/H1-2009	Influenza A H1N1 (swine)	The FluA-H1-2009 assay may detect influenza A H1 gene sequences of swine origin, that will be reported as Influenza A Virus (subtype H1-2009) or as Influenza A (No Subtype Found), depending on the strain and concentration present Certain strains of avian H1N2 may also be detected and reported as H1-2009
Chlamydia pneumoniae	*Chlamydia gallinacea*	The *C. pneumoniae* assay may amplify homologous sequences in the listed species

Interference Testing

A study was conducted to evaluate the potential for assay interference due to the presence of endogenous and exogenous substances that may be present in upper respiratory specimens. In addition, testing was also performed with representative on- and off-panel microorganisms at very high concentrations to evaluate the potential for competitive interference between analytes. Testing of each potentially interfering substance/microorganism was performed using contrived positive and negative specimens. The positive specimens contained representative on-panel analytes at concentrations equivalent to 3X their respective LoD (**Table 10**). The concentration of each potentially interfering substance tested was chosen to represent a high level that may be found in natural clinical specimens. The results of the study are summarized in **Tables 11-13**.

Table 10. Representative on-panel analytes used to evaluate assay interference

Assay	Analyte	Characteristics	Concentration (per mL) [1]
Adenovirus	Adenovirus B Type 3 (ZeptoMetrix 0810062CF)	Non-enveloped DNA virus	2.4 $TCID_{50}$
Chlamydia pneumoniae	*Chlamydia pneumoniae* AR-39 (ATCC 53592)	Gram negative bacterium	60 IFU
Seasonal Coronavirus	Coronavirus NL63 (ZeptoMetrix 0810228CF)	Enveloped RNA virus	0.0075 $TCID_{50}$
Human Rhinovirus/Enterovirus	Human Rhinovirus A1 ZeptoMetrix 0810012CFN	Non-enveloped RNA virus	0.63 $TCID_{50}$
Influenza B Virus	Influenza B virus B/Florida/02/06 (Victoria) (ZeptoMetrix 0810255CF)	Enveloped RNA virus	0.099 $TCID_{50}$

IFU: Inclusion Forming Units; $TCID_{50}$: Tissue Culture Infectious Dose-50%
[1] 3X LoD

Table 11. Results from testing the effect of endogenous substances on the performance of the SPOTFIRE R Panel

Potentially Interfering Substance	Concentration	Contrived Samples	Number of Positive Results by Assay												
			Adenovirus	Seasonal Coronavirus	Coronavirus SARS-CoV-2	Human Metapneumovirus	Human Rhinovirus / Enterovirus	Influenza A	Influenza A H1-2009	Influenza A H3	Influenza B Virus	*B. parapertussis*	*B. pertussis*	*C. pneumoniae*	*M. pneumoniae*
Whole human blood (sodium citrate)	10% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Human sputum/mucus	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Human genomic DNA	20 ng/µL	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	5/5	5/5	0/5	0/5	4/5 [1]	0/5	0/5	0/5	5/5	0/5	0/5	5/5	0/5

Results from the representative assays for which Contrived Positive Samples were prepared are shown in red
Contrived Positive Samples contained the representative on-panel analytes at 3X LoD
Contrived Negative Samples contained only the potentially interfering substance
[1] Initially, 1/3 samples was reported as negative; therefore, 2 additional replicates were tested, both of which produced the expected results for all analytes

None of the endogenous substances tested caused reproducible interference with the SPOTFIRE R Panel.

Table 12. Results from testing the effect of exogenous substances on the performance of the SPOTFIRE R Panel

Potentially Interfering Substance	Concentration	Contrived Samples	Number of Positive Results by Assay												
			Adenovirus	Seasonal Coronavirus	Coronavirus SARS-CoV-2	Human Metapneumovirus	Human Rhinovirus / Enterovirus	Influenza A	Influenza A H1-2009	Influenza A H3	Influenza B Virus	*B. parapertussis*	*B. pertussis*	*C. pneumoniae*	*M. pneumoniae*
Promethazine hyrdrochloride	3.3×10^{-4} mg/mL	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Acetaminophen (paracetamol)	1.5×10^{-1} mg/mL	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Acetylsalicylic acid (aspirin)	3.0×10^{-2} mg/mL	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	4/5 [1]	0/3	0/3	0/3	5/5	0/3	0/3	5/5	0/3
Ibuprofen	2.2×10^{-1} mg/mL	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	5/5	5/5	0/5	0/5	4/5 [1]	0/5	0/5	0/5	5/5	0/5	0/5	5/5	0/5
Albuterol sulfate	5.4×10^{-5} mg/mL	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Triple antibiotic ointment (neomycin/polymyxin B/bacitracin)	2% w/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Mucinex Severe Nasal Congestion Relief Clear & Cool Spray	1% v/v	Negative [2]	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Saline nasal spray (0.65% disodium phosphate phenylcarbinol, monosodium phosphate, benzalkonium chloride)	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Vicks VapoRub Cough Suppressant Topical Analgesic (4.8% camphor, 1.2% eucalyptus oil, 2.6% menthol)	1% w/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Vaseline pretoleum jelly (100% white petrolatum)	1% w/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Orajel (0.13% benzalkonium chloride, 20% benzocaine, 0.5% menthol, 0.15% zinc chloride)	2% w/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Chloraseptic Sore Throat Spray (1.4% phenol)	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Vicks Formula 44 DM	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

Potentially Interfering Substance	Concentration	Contrived Samples	Number of Positive Results by Assay												
			Adenovirus	Seasonal Coronavirus	Coronavirus SARS-CoV-2	Human Metapneumovirus	Human Rhinovirus / Enterovirus	Influenza A	Influenza A H1-2009	Influenza A H3	Influenza B Virus	*B. parapertussis*	*B. pertussis*	*C. pneumoniae*	*M. pneumoniae*
(0.67 mg/mL dextromethorphan hydrobromide, 13 mg/mL guaifenesin)		Positive [2]	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Phenylephrine hydrochoride	1% w/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Nasal spray (50 mg fluticasone propionate)	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	5/5	5/5	0/5	0/5	4/5 [1]	0/5	0/5	0/5	5/5	0/5	0/5	5/5	0/5
Sucrets Sore Throat (2.0 mg dyclonine hydrochloride/lozenge)	1% w/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Benadryl Allergy Liqui-gels (25 mg/capsule diphenhydramine hydrochloride)	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive [2]	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Zican Cold Remedy (galphimia glauca, luffa operculata, sabadilla)	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Cold-eeze (2.3% zinc gluconate)	1% w/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
HALLS lozenge (5 mg menthol/lozenge)	1% w/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Listerine Cool Mint (0.042% menthol, 0.064% thymol, 0.06% methyl salicylate, 0.092% eucalyptol)	6.5% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Copenhagen Snuff (tobacco)	1% w/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	5/5	5/5	0/5	0/5	4/5 [1]	0/5	0/5	0/5	5/5	0/5	0/5	5/5	0/5
Juice Head (30% polypropylene glycol, 70% vegetable glycerin)	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3

Results from the representative assays for which Contrived Positive Samples were prepared are shown in red
Contrived Positive Samples contained the representative on-panel analytes at 3X LoD
Contrived Negative Samples contained only the potentially interfering substance

[1] Initially, 1/3 samples was reported as negative; therefore, 2 additional replicates were tested, both of which produced the expected results for all analytes

[2] Initially, 1/3 samples was reported as invalid; the sample was retested and the results for all analytes were as expected

None of the endogenous substances tested caused reproducible interference with the SPOTFIRE R Panel.

Table 13. Results from testing the effect of substances associated with specimen collection on the performance of the SPOTFIRE R Panel

Potentially Interfering Substance	Concentration	Contrived Samples	Number of Positive Results by Assay												
			Adenovirus	Seasonal Coronavirus	Coronavirus SARS-CoV-2	Human Metapneumovirus	Human Rhinovirus / Enterovirus	Influenza A	Influenza A H1-2009	Influenza A H3	Influenza B Virus	*B. parapertussis*	*B. pertussis*	*C. pneumoniae*	*M. pneumoniae*
Rayon swab (Copan Diagnostics, Inc)	1 swab, 15 min [1]	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Nylon flocked swab (Copan Diagnostics, Inc.)	1 swab, 15 min [1]	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Polyester swab (Copan Diagnostics, Inc.)	1 swab, 15 min [1]	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Calcium alginate swab (Puritan)	1 swab, 15 min [1]	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	3/3	0/3	0/3	3/3	0/3	0/3	3/3	3/3
Cary-Blair Medium	90% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Dulbecco's Modified Eagles Medium (DMEM)	90% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
0.9% Normal Saline	90% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Remel MicroTest M4 Viral Transport Medium (VTM)	90% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Viral Preservative Medium (VPM)	90% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Phosphate Buffered Saline (PBS)	90% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
PrimeStore Molecular Transport Medium (MTM)	90% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive [2]	4/4	4/4	0/4	0/4	4/4	0/4	0/4	0/4	4/4	0/4	0/4	4/4	0/4
Stuart Transport Medium	90% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
eNAT Molecular Transport Medium	90% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3

Results from the representative assays for which Contrived Positive Samples were prepared are shown in red

Contrived Positive Samples contained the representative on-panel analytes at 3X LoD
Contrived Negative Samples contained only the potentially interfering species
[1] Swab immersed in matrix for 15 min prior to testing
[2] Initially, 1/3 samples was reported as invalid; therefore 2 additional replicates were tested and both produced the expected results for all analytes

None of the exogenous substances associated with collection of nasopharyngeal swab specimens tested were tested caused interference with the SPOTFIRE R Panel.

Table 14. Results from testing the effect of disinfectants and decontamination products on the performance of the SPOTFIRE R Panel

Potentially Interfering Substance	Concentration	Contrived Samples	Number of Positive Results by Assay												
			Adenovirus	Seasonal Coronavirus	Coronavirus SARS-CoV-2	Human Metapneumovirus	Human Rhinovirus / Enterovirus	Influenza A	Influenza A H1-2009	Influenza A H3	Influenza B Virus	*B. parapertussis*	*B. pertussis*	*C. pneumoniae*	*M. pneumoniae*
Bleach, 15 min incubation (sodium hypochlorite)	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
	2% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Bleach 4 hour incubation (sodium hypochlorite)	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Bleach 18.5 hour incubation (sodium hypochlorite)	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Ethanol	7% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Ammonium chloride disinfecting wipes	0.25-0.5 sq. inch/sample	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
DNAzap	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
RNaseZap	1% v/v	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive [1]	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3

Results from the representative assays for which Contrived Positive Samples were prepared are shown in red
Contrived Positive Samples contained the representative on-panel analytes at 3X LoD
Contrived Negative Samples contained only the potentially interfering species
[1] Initially 1/3 samples was reported as invalid due to an instrument error; the sample was retested and produced the expected result

None of the disinfectants or decontamination products that were tested caused interference with the SPOTFIRE R Panel. However, although bleach was not shown to have an adverse effect on test performance under the conditions of this study, a warning to avoid contact of test samples with bleach is included in the device labeling because of its known potential to cause nucleic acid degradation.

Table 15. Summary of results from testing for competitive interference with on-panel analytes

Potentially Interfering On-panel Microorganism	Concentration (copies per mL)	Contrived Samples	Number of Positive Results by Assay												
			Adenovirus	Seasonal Coronavirus	Coronavirus SARS-CoV-2	Human Metapneumovirus	Human Rhinovirus / Enterovirus	Influenza A	Influenza A H1-2009	Influenza A H3	Influenza B Virus	*B. parapertussis*	*B. pertussis*	*C. pneumoniae*	*M. pneumoniae*
Adenovirus A31	1.6×10^7	Negative	3/3 [1]	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Coronavirus 229E	1.5×10^7	Negative	0/3	3/3 [1]	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Enterovirus D68	7.8×10^7	Negative	0/3	0/3	0/3	0/3	3/3 [1]	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Parainfluenza Virus 3	8.0×10^6	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Respiratory Syncytial Virus A	1.5×10^7	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Bordetella pertussis	1.6×10^9	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3

Results from the representative assays for which Contrived Positive Samples were prepared are shown in red
Contrived Positive Samples contained the representative on-panel analytes at 3X LoD
Contrived Negative Samples contained only the potentially interfering species
[1] Expected positive result

No interference was observed with high concentrations of on-panel microbial species that may be present in nasopharyngeal swab specimens.

Table 16. Summary of results from testing for competitive interference with off-panel analytes

Potentially Interfering Off-panel Microorganism	Concentration (per mL)	Contrived Samples	Number of Positive Results by Assay												
			Adenovirus	Seasonal Coronavirus	Coronavirus SARS-CoV-2	Human Metapneumovirus	Human Rhinovirus / Enterovirus	Influenza A	Influenza A H1-2009	Influenza A H3	Influenza B Virus	*B. parapertussis*	*B. pertussis*	*C. pneumoniae*	*M. pneumoniae*
Cytomegalovirus	4.2 x 10^4 $TCID_{50}$	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Herpes Simplex Virus 1	9.0 x 10^6 $TCID_{50}$	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Haemophilus influenzae	8.3 x 10^8 CFU	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Staphylococcus aureus	7.4 x 10^8 CFU	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Streptococcus pneumoniae	2.5 x 10^7 CFU	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Streptococcus pyogenes	2.2 x 10^8 CFU	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3
Candida albicans	2.8 x 10^7 CFU	Negative	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		Positive	3/3	3/3	0/3	0/3	3/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3	0/3

CFU: Colony Forming Units; IFU: Tissue Culture Infectious Dose-50%
Results from the representative assays for which Contrived Positive Samples were prepared are shown in red
Contrived Positive Samples contained the representative on-panel analytes at 3X LoD
Contrived Negative Samples contained only the potentially interfering species

No interference was observed with off-panel microbial species that may be present in nasopharyngeal swab specimens.

4. Assay Reportable Range:

   Not applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Specimen Stability

   Viral Transport Medium
   The stability of most of the respiratory analytes targeted by the SPOTFIRE R Panel was demonstrated previously using simulated nasopharyngeal swab matrix in viral transport

medium under K103175, K110764 and K120267 for the BIOFIRE FilmArray Respiratory Panel (RP). *Bordetella parapertussis* and SARS-CoV-2 were not included in these studies but are biologically similar to other analytes on the panel (i.e., *B. pertussis* and other seasonal coronaviruses). The original specimen stability studies with the FilmArray RP are therefore applicable to support transport and storage claims for nasopharyngeal swab specimens in viral transport medium for use with the SPOTFIRE R Panel (i.e., 4 hours at 15-25 °C, 3 days at 2-8 °C or 30 days at < -15 °C).

0.9% Saline
A stability study was conducted with nasopharyngeal specimens in 0.9% saline under DEN200031 for the BIOFIRE FilmArray Respiratory Panel 2.1. Included in the study were all the analytes for this specimen type that are applicable to the SPOTFIRE R Panel. The results of the study therefore support the stability of the SPOTFIRE R Panel analytes in 0.9% saline (4 hours at 15-25 °C, 3 days at 2-8 °C or 30 days at < -15 °C).

6. Detection Limit:

Analytical Sensitivity
The Limit of Detection (LoD) for each analyte on the SPOTFIRE R Panel was determined with samples prepared using artificial nasopharyngeal swab matrix in Viral Transport Medium. A study showing that the formulation of the artificial matrix neither enhanced nor impaired assay performance is described in **Section VII B(2)**. The identity and concentration of each viral or bacterial stock used in the study was either certified by the commercial supplier or confirmed by quantitative real-time or digital PCR and standard methods. An initial titration of each viral or bacterial stock was performed to estimate the LoD, which was then confirmed by testing 20 replicates at the estimated LoD concentration and at one 10-fold lower dilution. The LoD was considered confirmed if ≥ 19/20 replicates at the estimated LoD concentration produced Positive results and ≤ 18/20 replicates at a 10-fold lower concentration were reported as Negative.

For efficiency, the LoD study was conducted by co-spiking multiple analytes into each sample. Therefore, additional testing was performed using a representative subset of target analytes to determine whether testing of multiple species in the same reaction had any effect test performance. Samples containing a single analyte were tested in replicates of 6 in parallel with samples containing the same analyte in addition to other viruses/bacteria on the SPOTFIRE R Panel. Overall, the results of the study showed that there was negligible difference in the analytical sensitivity of the SPOTFIRE R Panel from the presence of multiple target analytes in a sample (**Tables 17** and **18**).

Table 17. Summary of confirmed LoD values for nasopharyngeal swabs

Viruses				
Assay	**Strain/Type**	**Source**	**Limit of Detection (per mL)**	
Adenovirus	Species A Serotype 31	ZeptoMetrix 0810073CF	0.0041 $TCID_{50}$	100 copies
	Species B Serotype 3	ZeptoMetrix 0810062CF	0.8 $TCID_{50}$	840 copies
	Species C Serotype 2	WHO I.S. NIBSC 16-324	820 IU	820 copies
	Species D Serotype 37	ZeptoMetrix 0810119CF	0.011 $TCID_{50}$	450 copies
	Species E Serotype 4	ZeptoMetrix 0810070CF	0.018 $TCID_{50}$	10,000 copies
	Species F Serotype 41	ZeptoMetrix 0810085CF	0.014 $TCID_{50}$	100 copies
Seasonal Coronavirus	Coronavirus 229E	ATCC VR-740	0.65 $TCID_{50}$	11 copies
	Coronavirus HKU1	Clinical Specimen	--	18,000 copies
	Coronavirus OC43	ZeptoMetrix 0810024CF	0.016 $TCID_{50}$	63 copies
	Coronavirus NL63	ZeptoMetrix 0810228CF	0.0025 $TCID_{50}$	4.7 copies
Coronavirus SARS-CoV-2	USA-WA1/2020 (heat inactivated)	ATCC VR-1986HK	0.11 $TCID_{50}$	250 copies
Human Metapneumovirus	A1-16 Iowa 10/2003	ZeptoMetrix 0810161CF	3.2 $TCID_{50}$	240 copies
	B1-3 Peru2-2002	ZeptoMetrix 0810156CF	0.25 $TCID_{50}$	540 copies
	A2-27 Iowa A/2004	ZeptoMetrix 0810164CF	0.58 $TCID_{50}$	1,800 copies
	B2-18 IA18-2003	ZeptoMetrix 0810162CF	2 $TCID_{50}$	770 copies
Human Rhinovirus/ Enterovirus	Human Rhinovirus 1A	ZeptoMetrix 0810012CFN	0.21 $TCID_{50}$	1.1 copies
	Enterovirus D68 US/MO/14-18947	ATCC VR-1823	11 $TCID_{50}$	54 copies
Influenza A Virus Subtype H1-2009	H1N1 pdm A/ Michigan/45/15	ZeptoMetrix 0810538CF	0.82 $TCID_{50}$	340 copies
Influenza A Virus Subtype H3	H3N2 A/Hong Kong/ 4801/14	ZeptoMetrix 0810526CF	0.86 $TCID_{50}$	340 copies
Influenza B Virus	B/Florida/02/06 (Victoria)	ZeptoMetrix 0810037CF	0.033 $TCID_{50}$	160 copies

Viruses				
Assay	**Strain/Type**	**Source**	**Limit of Detection (per mL)**	
	B/Nevada/03/2011 (Victoria)	BEI NR-44023	1.6 $CEID_{50}$	4.3 copies
	B/Florida/04/06 (Yamagata)	ZeptoMetrix 0810255CF	0.4 $TCID_{50}$	32 copies
Parainfluenza Virus	Serotype 1	ZeptoMetrix 0810014CF	4.6 $TCID_{50}$	1,400 copies
	Serotype 2	ZeptoMetrix 0810015CF	14 $TCID_{50}$	160 copies
	Serotype 3	ZeptoMetrix 0810016CF	26 $TCID_{50}$	61 copies
	Serotype 4	ZeptoMetrix 0810060CF	200 $TCID_{50}$	1,100 copies
Respiratory Syncytial Virus	Type A 2006	ZeptoMetrix 0810040ACF	0.062 $TCID_{50}$	22 copies
	Type B 3/2015 Isolate #1	ZeptoMetrix 0810479CF	0.028 $TCID_{50}$	24 copies

Bacteria				
Assay	**Strain**	**Source**	**Limit of Detection (per mL)**	
Bordetella parapertussis	E595	ZeptoMetrix 0801462	40 CFU	--
Bordetella pertussis	A639	ZeptoMetrix 0801459	330 CFU	380 copies
Chlamydia pneumoniae	AR-39	ATCC 53592	20 IFU	140 copies
Mycoplasma pneumoniae	M129	ZeptoMetrix 0801579	10 CCU	2,100 copies

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

Table 18. Comparison of results from co-spiked and single spiked nasopharyngeal swab samples

Test Analyte	Co-spiked Species	Test Analyte Conc^n per mL	Positive	
			Co-spiked	Single Spike
Coronavirus 229E (ATCC VR-740)	Adenovirus D Human Metapneumovirus Enterovirus Parainfluenza Virus 4	13 $TCID_{50}$	6/6	6/6
		1.3 $TCID_{50}$	5/6	6/6
		0.13 $TCID_{50}$	4/6	1/6
		0.013 $TCID_{50}$	0/6	0/6
Influenza B Victoria B/Nevada/03/2011 (BEI NR-44023)	Adenovirus A Coronavirus NL63 Influenza A H3N2 Respiratory Syncytial Virus B *Bordetella pertussis*	160 $CEID_{50}$	6/6	6/6
		16 $CEID_{50}$	6/6	6/6
		1.6 $CEID_{50}$	5/6	6/6
		0.16 $CEID_{50}$	4/6	4/6
Parainfluenza Virus 1 (Zeptometrix 0810014CF)	Adenovirus C Coronavirus OC43 Human Metapneumovirus *Mycoplasma pneumoniae*	23 $TCID_{50}$	6/6	6/6
		2.3 $TCID_{50}$	6/6	6/6
		0.23 $TCID_{50}$	3/6	0/6
		0.023 $TCID_{50}$	0/6	0/6

$CEID_{50}$: Chicken Egg Infectious Dose-50%; $TCID_{50}$: Tissue Culture Infectious Dose-50%
Levels at or above the confirmed LoD are shown in red
Results obtained at the lowest level at which all replicates yielded positive results are highlighted in yellow

Inclusivity (Analytical Reactivity)

The inclusivity of the SPOTFIRE R Panel was evaluated using a combination of *in silico* analysis of publicly available sequence information and laboratory testing of contrived specimens containing viral and bacterial isolates that were selected to represent phylogenetic, geographic and temporal diversity.

In silico Analysis of Inclusivity

BLAST searches were conducted to obtain all available gene target sequences for each assay with the SPOTFIRE R Panel. *In silico* analysis of the inclusivity of the panel was performed using concatenated alignments of the nested primer sets for each target region. Mismatches to the primers were evaluated for their potential to affect detection of the target analyte based on their number and location within each primer sequence. *In silico* analysis of the SPOTFIRE R Panel primers showed that the target sequences are generally well conserved and that the assays are predicted to be robust to known mutations in these regions, with the ability to detect all or nearly all isolates of the targeted analytes.

In silico analysis of the ability to detect SARS-CoV-2 was performed using all available sequences in the GISAID (Global Initiative on Sharing Avian Influenza Data) database as of December 21, 2022. Included in the analysis were 11,989,970 sequences of which ≥ 99.99% were predicted to be detected by one or both SARS-CoV-2 assays within the SPOTFIRE R Panel, including sequences of all lineages, Variants of Concern and Variants of Interest identified to-date. Impaired sensitivity for detection by both SARS-CoV-2 assays was predicted for < 0.004% of sequences evaluated (427/11,989,970).

Laboratory Testing of Inclusivity

Contrived samples containing individual viruses and bacteria were prepared at approximately 3X LoD containing artificial matrix and tested in triplicate (unless noted in the tables below). All the viral and bacterial isolates tested were successfully detected (**Tables 19-32**).

Table 19. Strains of Adenovirus evaluated for inclusivity

Species	Source Identity	Type [Strain]	Concentration (copies/mL) [1]
A	**Zeptometrix 0810073CF [2]**	**31**	**200**
	ATCC VR-863	12 [Huie/Massachusetts]	600
	ATCC VR-19	18 [Washington DC/1954]	95
B	**Zeptometrix 0810062CF [2]**	**3**	**840**
	ATCC VR-7	7 [Gomen/California/1954]	2,500
	Zeptometrix 0810021CF	7a	2,500
	UIRF	7d/d2 [Iowa/2001]	2,500
	ATCC VR-12	11 [Slobitski/Massachusetts]	2,500
	ATCC VR-15	14 [De Wit/Netherlands/1955]	2,500
	ATCC VR-17	16 [CH. 79/Saudi Arabia/1955]	2,500
	ATCC VR-1833	21 [128/Saudi Arabia/1956]	2,500
	ATCC VR-716	34 [Compton/1972]	2,500
	ATCC VR-718	35 [Holden]	2,500
	ATCC VR-1602	50 [WaNAmsterdam/1988]	2,500
C	**WHO NIBSC 16/324 [2]**	**2**	**800**
	Zeptometrix 0810050CF	1	2,500
	Zeptometrix 0810020CF	5	2,500
	ATCC VR-6	6 [Tonsil 99]	2,500
D	**Zeptometrix 0810119CF [2]**	**37**	**450**
	Zeptometrix 0810069CF	8	1,400
	Zeptometrix 0810115CF	20 [KB]	1,400
E	**Zeptometrix 0810070CF [2]**	**4**	**10,000**
	ATCC VR-1572	4 [RI-67/Missouri/1952-1953]	30,000
	UIRF	4a [S. Carolina/2004]	30,000
F	**Zeptometrix 0810085CF [2]**	**41 [Tak]**	**100**
	NCPV 0101141v	40	1,400 [3]
	Zeptometrix 0810084CF	40	300
	ATCC VR-930	41 [Tak (73-3544)]	48 [4]

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD
[3] Not tested at lower levels
[4] 4/5 replicates reported positive; a higher level was not tested

Table 20. Strains of seasonal Coronavirus evaluated for inclusivity

Species	Source Identity [Strain]	Concentration (copies/mL) [1]
NL63	**Zeptometrix 0810228CF [2]**	**4.7**
	Amsterdam/2003 BEI NR-470	14
229E	**ATCC VR-740 [2]**	**11**
	Zeptometrix 0810229CF	33
HKU1	**BIOFIRE 03-0447 [Columbus OH, 2016] [2]**	**18,000 [3]**
	BIOFIRE 1-063 [South Carolina, 2010]	54,000
	BIOFIRE 04-0394 [Lyon, France, 2016]	54,000
	BIOFIRE 04-0394 [Lyon, France, 2016]	54,000
OC43	**Zeptometrix 0810024CF [2]**	**63**
	ATCC VR-759	190

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD
[3] 19/20 replicates reported positive

Table 21. Strains of SARS-CoV-2 evaluated for inclusivity

Source Identity	Strain	Concentration (copies/mL) [1]
ATCC VR-1986HK [2]	**USA-WA1/2020 (heat inactivated)**	**250 [3]**
ATCC VR-1991D	Hong Kong/VM20001061/2020	750
ATCC VR-1992D	2019-nCoV/Italy-INMI1	750
ATCC VR-1994D	Germany/BavPat1/2020	750
ATCC VR-3326D	USA/CA_CDC_5574/2020	750
BEI NR-52499	England/02/2020	750
BEI NR-52501	Singapore/2/2020	750
BEI NR-52503	USA-IL1/2020	750
BEI NR-52505	USA-AZ1/2020	750
BEI NR-52507	USA-CA3/2020	750
BEI NR-52510	Chile/Santiago_op4d1/2020	750
BEI NR-53518	New York-PV08410/2020	750
LGC SeraCare AccuPlex 0505-0298	Omicron B.1.1.529 Variant	750

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD
[3] 19/20 replicates reported positive

Table 22. Strains of Human Rhinovirus evaluated for inclusivity

Species	Source Identity	Type [Strain	Concentration (copies/mL) [1]
Rhinovirus A	**Zeptometrix 0810012CFN [2]**	**A1 [1A]**	**1.1**
	ATCC VR-1187	A77 [130-63]	3.3
	ATCC VR-1195	A85 [50-525-CV54]	3.3
	ATCC VR-1365	A34 [137-3]	3.3
	ATCC VR-1600	A57 [Ch47]	3.3
	ATCC VR-1601	A7 [68-CV11]	3.3
	ATCC VR-283	A16 [11757]	3.3
	ATCC VR-482	A2 [HGP]	11 [3]
Rhinovirus B	ATCC VR-1663	B17 [33342]	3.3 [4]
	ATCC VR-284	B14 [1059]	3.3
	ATCC VR-1950	B42 [56822]	3.3
	ATCC VR-483	B3 [FEB]	3.3
	ATCC VR-1137	B27 [5870]	3.3
	ATCC VR-1193	B83 [Baylor 7]	3.3

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD
[3] 1/3 replicates reported positive at 3.3 copies/mL
[4] 5/5 replicates reported positive

Table 23. Strains of Enterovirus evaluated for inclusivity

Species	Serotype	Source Identity	Strain	Concentration (copies/mL) [1]
Enterovirus D	**Enterovirus D68**	**ATCC VR-1823 [2]**	**US/MO/14-18947**	**54**
Enterovirus A	Enterovirus 71	ATCC VR-1432	71H	160
	Coxsackievirus 10	ATCC VR-168	NY/1950	160
Enterovirus B	Coxsackievirus A9	Zeptometrix 0810017CF	N/A	160
	Echovirus 11	Zeptometrix 0810023F	N/A	160
	Coxsackievirus B3	Zeptometrix 0810074CF	N/A	160
	Coxsackievirus B4	Zeptometrix 0810075CF	N/A	160
	Echovirus 6	Zeptometrix 0810076CF	N/A	160
	Echovirus 9	Zeptometrix 0810077CF	N/A	160
Enterovirus C	Coxsackievirus A24	ATCC VR-583	DN-19 TX/1963	160 [3]
	Coxsackievirus A21	ATCC VR-850	Kuykendall/CA/1952	160

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD
[3] 4/5 replicates reported positive; a higher level was not tested

Table 24. Strains of Human Metapneumovirus evaluated for inclusivity

Clade	Serotype	Source Identity	Strain	Concentration (copies/mL) [1]
A1	**16**	**Zeptometrix 0810161CF [2]**	**Iowa 10/2003**	**240**
	9	Zeptometrix 0810160CF	Iowa 3/2002	720
A2	**27**	**Zeptometrix 0810164CF [2]**	**Iowa 27/2004**	**1,800**
	20	Zeptometrix0810163CF	Iowa 14/2003	5,400
B1	**3**	**Zeptometrix 0810156CF [2]**	**Peru 2-2002**	**540**
	5	Zeptometrix 0810158CF	Peru 3/2003	1,600
B2	**18**	**Zeptometrix 0810162CF [2]**	**IA18-2003**	**770**
	4	Zeptometrix 0810157CF	Peru 1/2002	2,300
	8	Zeptometrix 0810159CF	Peru 6/2003	2,300
	Unknown	BEI NR-22232	N/A	2,300

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD

Table 25. Strains of Influenza A evaluated for inclusivity

Subtype	Source Identity	Strain	Concentration (copies/mL) [1]
H1N1pdm09	**Zeptometrix 0810538CF [2]**	**Michigan/45/15**	**340**
	BEI NR-19823	Netherlands/2629/2009	1,000
	BEI NR-42938	Georgia/F32551/2012	1,000
	BEI NR-44345	Hong Kong/H090-761-V1(0)/2009	1,000
	Zeptometrix 0810109CFJ	Canada/6294/2009	1,000
	Zeptometrix 0810165CF	California/07/2009	1,000
	Zeptometrix 0810166CF	Mexico/4108/2009	1,000
	Zeptometrix 0810249CF	Swine/NY/03/2009	1,000
H1N1 [3]	Zeptometrix 0810036CF	New Caledonia/20/1999	1,000
	Zeptometrix 0810036CFN	Solomon Islands/3/2006	1,000
	Zeptometrix 0810244CF	Brisbane/59/2007	1,000
	ATCC VR-333	Swine/Iowa/15/1930	1,000
	ATCC VR-897	Swine/A/New Jersey/8/76	1,000
	ATCC VR-99	Swine/1976/1931	1,000
H3N2	**Zeptometrix 0810526CF [2]**	**Hong Kong/4801/14**	**340**
	ATCC VR-544	Hong Kong/8/1968	1,000
	ATCC VR-547	Aichi/2/1968	3,400 [4]
	ATCC VR-776	Alice	1,000
	ATCC VR-810	Port Chalmers/1/1973	1,000 [5]
	ATCC VR-822	Victoria/3/1975	1,000
	Zeptometrix 0810138CF	Brisbane/10/2007	1,000
	Zeptometrix 0810238CF	Texas/50/2012	1,100 [5]
	Zeptometrix 0810252CF	Wisconsin/67/2005	1,200
H1N2 [6]	BEI NR-3478	Recombinant: Kilbourne F63 A/NWS/1934 x A/Rockefeller Institute/5/1957 (NA)	74
H10N7 [6]	BEI NR-2765	Chicken/Germany/N/49	74
H2N2 [3]	BEI NR-2775	Japan/305/1957	2.2
H5N3 [3]	BEI NR-9682	Duck/Singapore/645/97	2.2

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD
[3] Reported as Influenza A Virus Positive (No Subtype Identified)
[4] 3/5 replicates reported positive for Influenza A Virus A/H3 at 1,000 copies/mL
[5] 4/5 replicates reported positive for Influenza A Virus A/H3; a higher level was not tested
[6] Reported as Uncertain: Influenza A Virus

Table 26. Strains of Influenza B evaluated for inclusivity

Lineage	Source Identity	Strain	Concentration (copies/mL) [1]
Yamagata	**Zeptometrix 0810255CF [2]**	**Florida/04/06**	**32**
	Zeptometrix 0810239CF	2/Massachusetts/2012	96
	Zeptometrix 0810241CF	1/Wisconsin/2010	96
	Zeptometrix 0810256CF	07/Florida/2004	96
Victoria	**Zeptometrix 0810037CF [2]**	**Florida/02/06**	**160**
	BEI NR-44023 [2]	**B/Nevada/03/2011**	**4.3**
	ATCC VR-823	5/Hong Kong/1972	13
	CDC 2005743348	1/Ohio/2005	13
	Zeptometrix 0810258CF	2506/Malaysia/2004	13
Unknown	ATCC VR-101	Lee/1940	13
	ATCC VR-102	Allen/1945	13 [3]
	ATCC VR-103	GL/1739/1954	13
	ATCC VR-295	2/Taiwan/1962	13
	ATCC VR-296	1/Maryland/1959	13 [3]
	ATCC VR-786	Brigit/Russia/1969	13 [3]

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD
[3] 4/5 replicates reported positive; a higher level was not tested

Table 27. Strains of Parainfluenza Virus evaluated for inclusivity

Serotype	Source Identity	Strain	Concentration (copies/mL) [1]
Parainfluenza Virus 1	**Zeptometrix 0810014CF [2]**	**N/A**	**710**
	ATCC VR-94	C-35/1957	2,100
	BEI NR-48680	FRA/29221106/2009	2,100
Parainfluenza Virus 2	**Zeptometrix 0810015CF [2]**	**N/A**	**160**
	ATCC VR-92	Greer/1955	480 [3]
Parainfluenza Virus 3	**Zeptometrix 0810016CF [2]**	**N/A**	**21**
	ATCC VR-93	C-243/1957	210 [4]
	BEI NR-3233	NIH 47885 Wash/47885/57	63
Parainfluenza Virus 4A	**Zeptometrix 0810060CF [2]**	**N/A**	**570**
	ATCC VR-1378	M-25/1958	1,700
	Zeptometrix 0810060CF	Unknown	1,700
Parainfluenza Virus 4B	ATCC VR-1377	CH-19503/1962	1,700
	Zeptometrix 0810060BCF	Unknown	1,700

N/A: Not available
[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD
[3] 4/5 replicates reported positive; a higher level was not tested
[4] 2/5 replicates reported positive at 63 copies/mL

Table 28. Strains of Respiratory Syncytial Virus evaluated for inclusivity

Subtype	Source Identity	Strain	Concentration (copies/mL) [1]
A	**Zeptometrix 0810040ACF [2]**	**2006**	**22**
	ATCC VR-26	Long/Maryland/1956	66
	ATCC VR-1540	A2/Melbourne/1961	66
	Zeptometrix 0810474CF	2/2015/ Isolate #2	66
	Zeptometrix 0810452CF	12/2014 Isolate #2	66
B	**Zeptometrix 0810479CF [2]**	**3/2015 Isolate #1**	**24**
	Zeptometrix 0810040CF	Ch-93 (18)-18	72
	ATCC VR-1400	WV/14617/1985	72
	ATCC VR-955	9320/Massachusetts/1977	72
	ATCC VR-1580	18537/WashingtonDC/1962	72
	Zeptometrix 0810451CF	11/2014 Isolate #2	72

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD

Table 29. Strains of *B. parapertussis* evaluated for inclusivity

Source Identity	Strain	Concentration (per/mL) [1]
Zeptometrix 0801462 [2]	**E595**	**41 CFU**
ATCC 9305	517	230 cells
ATCC 53892	PT28G	15 cells
ATCC 53893	PT 26/28G	17 cells
ATCC 15237	NCTC 10853	83 CFU
ATCC 15311	NCTC 5952	13 cells
ATCC BAA-587	12822/Germany/1993	120 CFU
Zeptometrix 0801461	A747	4.4 CFU
Zeptometrix 0801643	C510	200 CFU
Zeptometrix 0801644	E838	340 CFU [3]

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD
[3] 4/5 replicates reported positive; a higher level was not tested

Table 30. Strains of *B. pertussis* evaluated for inclusivity

Source Identity	Strain	Concentration (copies/mL) [1]
Zeptometrix 0801459 [2]	**A639**	**380**
ATCC 10380	10-536	1,100 [3]
ATCC 51445	CNCTC Hp 12/63 [623]	1,100
ATCC 8467	F	1,100
ATCC 9340	5 [17921]	1,100
ATCC 9797	18323 [NCTC 10739]	1,100
ATCC BAA-1335	MN2531	1,100
ATCC BAA-589	Tohama	1,100
Zeptometrix 0801460	E431	1,100

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD
[3] 5/5 replicates reported positive

Table 31. Strains of *C. pneumoniae* evaluated for inclusivity

Source Identity	Strain	Concentration (copies/mL) [1]
ATCC 53592 [2]	**AR-39**	**140**
ATCC VR-1310	CWL-029	420
ATCC VR-1356	TWAR strain 2023	420
ATCC VR-1360	CM-1	420
ATCC VR-1435	J-21	420
ATCC VR-1452	A03	420
ATCC VR-2282	TWAR strain, TW-183	420

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD

Table 32. Strains of *M. pneumoniae* evaluated for inclusivity

Subtype	Source Identity	Strain	Concentration (copies/mL) [1]
1	**Zeptometrix 0810159 [2]**	**M129**	**2,100**
	ATCC 29085	PI 1428	6,300
	ATCC 29342	M129-B7	6,300
2	ATCC 15492	Mac	6,300
	ATCC 15531-TTR	FH strain of Eaton Agent [NCTC 10119)	6,300
Unknown	ATCC 15293	M52	6,300
	ATCC 15377	Bru	6,300
	ATCC 39505	Mutant 22	6,300
	ATCC 49894	UTMP-10P	6,300

[1] Confirmed LoD concentration or lowest concentration at which all replicates reported positive if < 20 replicates tested
[2] Isolate used to determine the LoD

7. Assay Cut-Off:

DNA melt curve analysis is used to determine the presence or absence of the specific amplification product(s) corresponding to each analyte within the SPOTFIRE R Panel. The Melt Detector software analyzes each well of the second stage PCR array independently. A series of mathematical tests is applied to each fluorescence curve to discriminate target-specific amplicon from system noise and non-specific amplification products, taking into account both system variation and known sequence variation within the amplified regions targeted by the assay. The Melt Detector algorithm was developed by tuning against a large data set comprised of both typical and atypical melt curves that were assigned as positive or negative by manual review performed by expert users. The tuning process optimized the sensitivity and specificity of the algorithm in comparison to manual result interpretation and included optimization of the order of procedural steps and the respective threshold for each parameter.

For each assay within the SPOTFIRE R Panel, the expected melting temperature (mean Tm) was determined empirically using the most common sequence variants. The acceptable range around the mean was then determined through a combination of empirical testing of known sequence variants and mathematical modeling. Reference data obtained from analysis of templates with known sequences were used to estimate additional sources of system variability, including the SPOTFIRE instrument and reagents. The melt range parameters for each analyte were validated in the subsequent Analytical and Clinical Studies.

8. Accuracy (Instrument):

Refer to **Section VII (C) Clinical Studies**.

9. Carry-Over:

The potential for sample-to-sample cross-contamination during operation of the SPOTFIRE System was evaluated by testing an alternating series of positive and negative contrived samples using the same Pouch Loading Station. The positive samples comprised high levels of representative on-panel analytes in artificial matrix. Negative samples comprised artificial matrix alone. No unexpected positive results were observed from a total of 15 negative samples that were tested. These results demonstrated that there is an acceptable, low likelihood of cross-contamination between samples when the SPOTFIRE R Panel is performed according to the instructions for use.

Table 33. Results from evaluation of the potential for cross-contamination on the BIOFIRE SPOTFIRE System

Analyte	High Positive Target Level		SPOTFIRE R Panel Result	
	Concentration (per mL)	Multiple of LoD	Positive	Negative
Chlamydia pneumoniae	290 IFU	1.5×10^5	5/5	0/15
Enterovirus	1.6×10^7 $TCID_{50}$	1.5×10^6	5/5	0/15
Influenza A H3N2	7.2×10^4 $TCID_{50}$	8.4×10^4	5/5	0/15

IFU: Inclusion Forming Units; $TCID_{50}$: Tissue Culture Infectious Dose-50%

B Comparison Studies:

1. Method Comparison with Predicate Device:

Refer to **Section VII (C) Clinical Studies**.

2. Matrix Comparison:

Artificial vs Natural Matrix
The SPOTFIRE R Panel is intended to detect multiple viral and bacterial analytes in nasopharyngeal swab specimens. The high frequency of positive samples for at least one analyte makes it impractical to use natural clinical matrices for analytical validation studies for which large volumes of negative matrix are required. To address this, artificial matrix was used for most of the analytical testing and therefore an additional study was conducted to demonstrate that the analytical sensitivity of the SPOTFIRE R Panel was similar with samples prepared in artificial and natural specimen matrices, as described below.

Artificial nasopharyngeal swab (aNS) matrix comprised glycerol, porcine mucin, human whole blood, phosphate buffered saline and saline diluted in transport medium (Viral Transport Medium [VTM]). Testing was performed using a representative subset of analytes targeted by the SPOTFIRE R Panel, including both DNA and RNA viruses. Ten-fold serial dilutions of each analyte were prepared in artificial matrix and, in parallel, in natural clinical matrix obtained from asymptomatic donors. Prior to conducting the study, the natural clinical

specimens were tested to verify that none of the on-panel analytes were present. A summary of the results is presented in **Table 34**.

Positive results were obtained for all replicates with each analyte at target levels equal to or above the claimed LoD except for Adenovirus in natural clinical matrix for which only 4/6 replicates (67%) were reported positive at the claimed LoD concentration. However, amplification curve characteristics for Adenovirus were similar between positive replicates in both the artificial and natural nasopharyngeal swab matrices at the LoD target level for this analyte and all replicates in both matrices were positive at a 10-fold higher concentration, suggesting that the analytical sensitivity of the assay with the two matrices is similar.

Overall, the results of the matrix equivalency study showed that the use of artificial matrix does not enhance or impair device performance and is acceptable for analytical validation of assay performance.

Table 34. Comparison of analytical sensitivity in artificial and natural nasopharyngeal swab matrices

Analyte	Strain	Source	Units	Per mL	Positive/Tested (%)	
					aNS	nNS
Adenovirus B	Type 3	ZeptoMetrix 0810062CF	$TCID_{50}$	8.2	5/5 (100)	6/6 (100)
				0.82 [1]	6/6 [1] (100)	4/6 [1] (67)
				0.082	1/6 (17)	1/6 (17)
				0.0082	0/6 (0)	0/6 (0)
Influenza A	Subtype H1-2009 Michigan/45/15	ZeptoMetrix 0810538CF	$TCID_{50}$	8.2	5/5 (100)	6/6 (100)
				0.82 [1]	6/6 (100)	6/6 (100)
				0.082	1/6 (17)	2/6 (33)
				0.0082	0/6 (0)	0/6 (0)
Influenza B	Florida/02/06	ZeptoMetrix 0810037CF	$TCID_{50}$	3.3	5/5 (100)	6/6 (100)
				0.33	6/6 (100)	6/6 (100)
				0.033 [1]	6/6 (100)	6/6 (100)
				0.0033	0/6 (0)	1/6 (17)
SARS-CoV-2	USA-WA1/2020 (heat inactivated)	ATCC VR-1986HK	copies	5,000	5/5 (100)	6/6 (100)
				500 [2]	6/6 (100)	6/6 (100)
				50	2/6 (33)	2/6 (33)
				5	0/6 (0)	0/6 (0)

$TCID_{50}$: Tissue Culture Infectious Dose-50%
aNS: artificial nasopharyngeal swab matrix; nNS: natural nasopharyngeal swab matrix
Results from target levels below the claimed LoD of each analyte are shaded
[1] LoD target level (1X)
[2] 2X LoD

Fresh vs Frozen Samples
A study was performed with the BIOFIRE FilmArray Respiratory Panel under K160068 using fresh and frozen nasopharyngeal swabs which showed that there were no adverse effects from freezing and thawing of specimens. Because of the similarities between the BIOFIRE FilmArray Respiratory Panel and the SPOTFIRE R Panel in terms of the targeted analytes, technology and reagent formulations, these data are considered acceptable to support testing of archived, frozen specimens in the Clinical Study to evaluate the performance of the SPOTFIRE R Panel.

Transport Media Equivalency
A study was conducted to evaluate the compatibility of the SPOTFIRE R Panel with alternative transport media, including 0.9% normal saline, Hank's Balanced Salt Solution (Gibco), BD Universal Viral Transport (UVT) medium (BD Medical), and Remel MicroTest M4RT (Thermo Fisher). Testing was performed using contrived samples in artificial nasopharyngeal swab matrix containing representative analytes (including bacteria and DNA and RNA viruses) at concentrations spanning the claimed analytical LoD (5X, 1X and 0.2X LoD). For the purposes of the study, the test analytes were prepared in the form of two panels, each of 5 on-panel species. The results obtained with each of the alternative transport media were compared to those obtained using Viral Transport Medium (VTM) as a control and are summarized in **Table 35**.

The results of the study met the acceptance criteria in all cases thereby demonstrating the compatibility of the SPOTFIRE R Panel with 0.9% Saline, Hanks Balanced Salt Solution, BD UVT medium and M4RT transport medium.

Table 35. Comparison of alternative transport media for use with the BIOFIRE SPOTFIRE R Panel

Analyte Panel 1								
Analyte	**Level (X LoD)**	**Percent Positive**						
		Run 1		**Run 2**			**Run 3**	
		VTM	**UVT**	**VTM**	**Saline**	**HBSS**	**VTM**	**M4RT**
Adenovirus	5	100	100	100	100	100	100	100
	1	100	100	100	100	100	100	100
	0.2	100	100	78	89	100	89	100
Coronavirus NL63	5	100	100	100	100	100	100	100
	1	96	96	100	100	100	96	96
	0.2	44	56	48	78	56	11	89
Human Rhinovirus/ Enterovirus	5	100	100	100	100	100	100	100
	1	100	100	100	100	100	100	100
	0.2	89	100	100	100	100	89	100
Influenza B	5	100	100	100	100	100	100	100
	1	100	100	100	100	100	100	100
	0.2	100	100	100	100	100	89	100
Bordetella pertussis	5	100	100	100	100	100	100	100
	1	100	100	100	100	96	96	96
	0.2	56	67	67	78	78	33	33

Analyte Panel 2							
Analyte	**Level (X LoD)**	**Percent Positive**					
		Run 1		**Run 2**			
		VTM	**UVT**	**VTM**	**Saline**	**HBSS**	**M4RT**
Influenza A	5	100	100	100	100	100	100
	1	100	100	100	100	100	100
	0.2	100	100	100	100	100	100
Human Metapneumovirus	5	100	100	100	100	100	100
	1	100	100	100	96	100	100
	0.2	89	89	67	100	89	89
Parainfluenza Virus 2	5	100	100	100	100	100	100
	1	100	100	100	100	100	100
	0.2	78	67	100	67	100	89
Respiratory Syncytial Virus	5	100	100	100	100	100	100
	1	100	100	100	100	100	100
	0.2	100	100	100	100	100	100
Chlamydia pneumoniae	5	100	100	100	100	100	100
	1	100	100	100	100	100	100
	0.2	78	56	67	67	56	100

HBSS: Hank's Balanced Salt Solution (Gibco); M4RT: Remel MicroTest M4RT (Thermo Fisher); Saline: 0.9% normal saline; UVT: Universal Viral Transport (BD); VTM: Viral Transport Medium; LoD Limit of Detection

C Clinical Studies:

1. Clinical Sensitivity:

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

Prospectively Collected Specimens
A Clinical Study with prospectively collected specimens was conducted at five sites (4 U.S. and 1 ex-U.S.), including adult and pediatric emergency departments/urgent care centers, that were considered representative of the intended use settings for the BIOFIRE SPOTFIRE System and SPOTFIRE R Panel. The specimens included in the study were either collected under Informed Consent (if required by the participating institution), and with parental permission for minors < 18 years of age or were leftover (residual) samples from standard of care testing. The inclusion and exclusion criteria for the study, which spanned the period between December 2020 to June 2021, are summarized in **Table 36**. Aliquots of each specimen were prepared for testing with the SPOTFIRE R Panel and the applicable comparator method(s). All aliquots of nasopharyngeal swab specimens for comparator testing were stored at -70 °C. At each of the study sites, specimen enrollment began prior to the availability of BIOFIRE SPOTFIRE instruments. During this period, the specimen aliquots for testing with the SPOTFIRE R Panel were frozen at -70 °C (refer to Matrix Comparison in **Section VII (B)(2)**).

Table 36. Inclusion and exclusion criteria for the Prospective Clinical Study

Specimens Collected Under Informed Consent [1, 2]		
Inclusion Criteria	Respiratory Illness	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 specimen	
	Subject's healthcare provider determined that specimen collection represented an unacceptable healthcare risk	
Residual Specimens [3]		
Inclusion Criteria	Respiratory Illness	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] Includes specimens from minors collected with parental consent
[3] 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 description of the comparator methods used to establish the performance of the SPOTFIRE R Panel is provided in **Table 37**.

Table 37. Comparator methods used to characterize prospectively collected and archived clinical specimens

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

[1] Not detected by Multi-Analyte Panel-1 comparator
[2] Due to reduced sensitivity of the Multi-Analyte Panel-1 for influenza B, Multi-Analyte Panel-2 was used as the comparator for this analyte
[3] Separate results reported for coronaviruses 229E, HKU1, NL63 and OC43, as well as parainfluenza virus serotypes 1 - 4

A total of 1215 nasopharyngeal were initially enrolled in the Prospective Clinical Study, of which 95 were excluded from the analysis of performance for the reasons listed in **Table 38**. In addition, some specimens were excluded from the analysis of performance for one or more specific analytes due to improper storage of sample aliquots or to failure or inappropriate conduct of the comparator method and inability to perform retesting due volume constraints. All nasopharyngeal swabs included in the analysis of performance were collected in viral transport medium (VTM).

Table 38. 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 not be performed within the specified time	1
Specimen handling error	4
Specimen not aliquoted within the specified time	8
Invalid SPOTFIRE R Panel result	38
Specimen not received for comparator testing	29
Use of expired SPOTFIRE R Panel reagents [1]	11
Total Excluded	**95**
Total Included	**1120**

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

A summary of the demographic information of the subjects included in the Prospective Clinical Study is provided in **Table 39**.

Table 39. Demographics of subjects included in the Prospective Clinical Study

Category		Number	Percent
Sex	Male	587	52.4
	Female	533	47.6
	Total	**1120**	**100**
Age (years)	≤ 5	457	40.8
	6 – 18	258	23.0
	19 – 40	160	14.3
	41 – 60	147	13.1
	≥ 61	98	8.8
	Total	**1120**	**100**

Archived Specimens

Several analytes included on the SPOTFIRE R Panel were not encountered during the Prospective Clinical Study in sufficient numbers to demonstrate system performance. Therefore, the Prospective Clinical Study was supported by additional testing that was performed at the four U.S. study sites on frozen, archived specimens obtained from various clinical laboratories from around the world. These specimens were selected for inclusion in the study based on their microbial content as originally determined by the source laboratory and were stored frozen prior to testing. The microbial content of the archived specimens was confirmed using the same molecular comparator methods as for the prospectively collected specimens (**Table 37**). A mimimum sample volume of 650 µL was required for confirmatory testing.

A total of 562 archived nasopharyngeal swabs was obtained, of which 20 were excluded from the analysis of performance due to invalid SPOTFIRE R Panel results or to invalid or missing comparator test results (**Table 40**).

Table 40. Summary of data exclusions from the testing archived clinical specimens

Rationale for Exclusion	Nasopharyngeal Swab
Invalid BIOFIRE R/ST Panel Result	12
Invalid or missing comparator test result	8
Total	**20**

A summary of the demographic information of the subjects included in the Clinical Study with preselected, archived specimens is provided in **Table 41**.

Table 41. Demographics of subjects included in the Clinical Study with preselected, archived specimens

Category		Number	Percent
Sex	Male	254	46.9
	Female	185	34.1
	Unknown	103	19.0
	Total	**542**	**100**
Age (years)	≤ 5	234	43.2
	6 – 18	98	18.1
	19 – 40	36	6.6
	41 – 60	35	6.5
	≥ 61	39	7.2
	Unknown	100	18.5
	Total	**542**	**100**

Clinical Performance

A summary of the results from testing prospectively collected and archived nasopharyngeal swabs is shown in **Table 42**. Positive Percent Agreement (PPA) ranged from 96.3-100% for prospectively collected specimens and from 96.0-100% for archived specimens, depending on the analyte, whereas Negative Percent Agreement (NPA) ranged from 90.6-100% for prospectively collected specimens and from 96.7-100% for archived specimens. The only analyte for which NPA was < 95% was Human Rhinovirus/Enterovirus with prospectively collected specimens. However, additional testing provided evidence for the presence of Human Rhinovirus/Enterovirus in 54/72 prospectively collected specimens (75.0%) with discordant positive SPOTFIRE R Panel results and therefore the low NPA with this analyte in the prospective study was considered acceptable.

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

Table 42. Clinical performance of the SPOTFIRE R Panel: prospectively collected and archived specimens

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

Viruses					
Assay	**Study**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
	Archived	100 (90.6-100)	37/37	98.4 (96.8-99.2)	440/447

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

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

[1] 54/72 specimens with "false positive" results for Human Rhinovirus/Enterovirus (75.0%) were reported positive for these analytes upon retesting with the same FDA-cleared comparator (1), by testing with either an alternative FDA-cleared assay (44) or with PCR followed by bidirectional sequencing (8), or both (1)

Table 43 shows further stratification of the results from testing prospectively collected nasopharyngeal specimens according to the temperature of storage prior to analysis. Most specimens were held at either 4 °C (n = 844; 75.3%) or room temperature (n = 17; 1.5%) while the remainder (n = 259; 23.1%) were frozen (≤ -70 °C). There were no notable differences in performance based on the temperature of specimen storage.

Table 43. Stratification of results obtained with prospectively collected nasopharyngeal swabs based on the temperature of specimen storage prior to testing

Viruses					
Analyte	**Storage Condition**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
Adenovirus	Fresh	96.4 (82.3-99.4)	27/28	98.1 (96.9-98.8)	814/830
	Frozen	100 (56.6-100)	5/5	96.8 (93.9-98.4)	244/252
	Combined	97.0 (84.7-99.5)	32/33	97.8 (96.7-98.5)	1058/1082
Seasonal Coronavirus	Fresh	99.0 (94.4-99.8)	97/98	98.6 (97.4-99.2)	749/760
	Frozen	100 (51.0-100)	4/4	99.2 (97.2-99.8)	251/253
	Combined	99.0 (94.7-99.8)	101/102	98.7 (97.8-99.2)	1000/1013
Coronavirus SARS-CoV-2	Fresh	96.8 (83.8-99.4)	30/31	99.8 (99.1-99.9)	827/829
	Frozen	97.6 (87.7-99.6)	41/42	98.1 (95.2-99.2)	204/208
	Combined	97.3 (90.6-99.2)	71/73	99.4 (98.7-99.7)	1031/1037
Human Metapneumovirus	Fresh	N/A	N/A	100 (99.6-100)	858/858
	Frozen	100 (20.7-100)	1/1	100 (98.5-100)	256/256
	Combined	100 (20.7-100)	1/1	100 (99.7-100)	1114/1114
Rhinovirus/Enterovirus	Fresh	99.3 (97.5-99.8)	290/292	89.9 (87.2-92.1)	509/566
	Frozen	98.2 (90.6-99.7)	55/56	92.5 (88.1-95.4)	186/201
	Combined	99.1 (97.5-99.7)	345/348	90.6 [1] (88.3-92.5)	695/767
Influenza A	Fresh	N/A	N/A	100 (99.6-100)	858/858
	Frozen	N/A	N/A	100 (98.5-100)	257/257
	Combined	N/A	N/A	100 (99.7-100)	1115/1115
Influenza A H1-2009	Fresh	N/A	N/A	100 (99.6-100)	858/858
	Frozen	N/A	N/A	100 (98.5-100)	257/257
	Combined	N/A	N/A	100 (99.7-100)	1115/1115

Viruses					
Analyte	**Storage Condition**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
Influenza A H3	Fresh	N/A	N/A	100 (99.6-100)	858/858
	Frozen	N/A	N/A	100 (98.5-100)	257/257
	Combined	N/A	N/A	100 (99.7-100)	1115/1115
Influenza B	Fresh	N/A	N/A	100 (99.6-100)	860/860
	Frozen	N/A	N/A	100 (98.5-100)	250/250
	Combined	N/A	N/A	100 (99.7-100)	1110/1110
Parainfluenza Virus	Fresh	97.8 (92.5-99.4)	91/93	98.8 (97.8-99.4)	756/764
	Frozen	100 (56.6-100)	5/5	99.2 (97.2-99.8)	250/252
	Combined	98.0 (92.9-99.4)	96/98	98.9 (98.1-99.4)	1006/1017
Respiratory Syncytial Virus	Fresh	96.0 (80.5-99.3)	24/25	99.8 (99.1-99.9)	831/833
	Frozen	100 (34.2-100)	2/2	100 (98.5-100)	255/255
	Combined	96.3 (81.7-99.3)	26/27	99.8 (99.3-100)	1086/1088

Bacteria					
Analyte	**Study**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
Bordetella parapertussis	Fresh	N/A	N/A	100 (99.6-100)	860/860
	Frozen	N/A	N/A	100 (98.5-100)	250/250
	Combined	N/A	N/A	100 (99.7-100)	1110/1110
Bordetella pertussis	Fresh	N/A	N/A	100 (99.6-100)	858/858
	Frozen	N/A	N/A	100 (98.5-100)	257/257
	Combined	N/A	N/A	100 (99.7-100)	1115/1115
Chlamydia pneumoniae	Fresh	N/A	N/A	100 (99.6-100)	858/858
	Frozen	N/A	N/A	100 (98.5-100)	257/257

Bacteria					
Analyte	**Study**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
	Combined	N/A	N/A	100 (99.7-100)	1115/1115
Mycoplasma pneumoniae	Fresh	N/A	N/A	100 (99.6-100)	858/858
	Frozen	N/A	N/A	100 (98.5-100)	257/257
	Combined	N/A	N/A	100 (99.7-100)	1115/1115

Fresh: Storage at ambient temperature or refrigerated; Frozen: Storage at ≤ -70 °C
PPA: Positive Percent Agreement; NPA: Negative Percent Agreement: 95% CI: 95% score confidence interval; TP: True Positive; FP: False Positive; False Negative; True Negative (all as determined with respect to the comparator); N/A: Not Applicable

[1] 54/72 specimens with "false positive" results for Human Rhinovirus/Enterovirus (75.0%) were reported positive for these analytes upon retesting with the same FDA-cleared comparator (1), by testing with either an alternative FDA-cleared assay (44) or with PCR followed by bidirectional sequencing (8), or both (1)

Coinfections

The SPOTFIRE R Panel reported infections due to a single analyte from 51.0% of nasopharyngeal swab specimens (551/1120) in the Prospective Clinical Study, and coinfection with two or more analytes from 9.5% of specimens (106/1120) (**Table 44**). Among the coinfected specimens, 90 (84.9%) were positive for two analytes and 16 (15.1%) were positive for three or more using the SPOTFIRE R Panel.

Table 44. Coinfections reported by the SPOTFIRE R Panel in the Prospective Clinical Study

Analyte 1	Analyte 2	Analyte 3	Analyte 4	Number of Specimens		SPOTFIRE R Panel False Positive Results [1]
				Total	False Positive	
Adenovirus	hMPV	hR/EV	PIV	1	1	Adenovirus, PIV
Adenovirus	Seasonal CoV	hR/EV		6	3	Adenovirus (2), hR/EV (1)
Adenovirus	Seasonal CoV	RSV		1	1	Seasonal CoV
Adenovirus	hR/EV	PIV		1	0	--
Seasonal CoV	hR/EV	PIV		4	4	Seasonal CoV (1), hR/EV (2), PIV (2)
SARS-CoV-2	hR/EV	PIV		1	0	--
hR/EV	PIV	RSV		2	2	hR/EV (2), PIV (1), RSV (1)
Adenovirus	Seasonal CoV			5	1	Adenovirus
Adenovirus	hR/EV			19	13	Adenovirus (11), hR/EV (5)
Adenovirus	PIV			4	2	Adenovirus (2)
Adenovirus	RSV			2	2	Adenovirus (2)
Seasonal CoV	hR/EV			11	5	hR/EV (3), Seasonal CoV (2)
Seasonal CoV	PIV			9	1	Seasonal CoV
Seasonal CoV	RSV			1	0	--
SARS-CoV-2	hR/EV			9	5	SARS-CoV-2 (1), hR/EV (5)
SARS-CoV-2	RSV			1	0	--
hR/EV	PIV			20	8	hR/EV (6), PIV (3)
hR/EV	RSV			8	5	hR/EV (4), RSV (1)
PIV	RSV			1	0	--
Total				**106**	**53**	**62/229 [2]**
Total Quadruple Infections				**1**	**1**	**2/4 [2]**
Total Triple Infections				**15**	**10**	**13/45 [2]**
Total Double Infections				**90**	**42**	**47/180 [2]**

hMPV: Human Metapneumovirus; hR/EV: Human Rhinovirus/Enterovirus; PIV: Parainfluenza Virus; Seasonal CoV: Seasonal Coronavirus; RSV: Respiratory Syncytial Virus

[1] Based on comparator test results

[2] Number false positive/total

Four specimens were positive for two analytes (i.e, coinfected) by the comparator methods but were reported as negative for one of the analytes using the SPOTFIRE R Panel (**Table 45**).

Table 45. Coinfections identified in nasopharyngeal swabs by the comparator methods not reported by the SPOTFIRE R Panel

Analyte 1	Analyte 2	Number of Specimens	SPOTFIRE R Panel False Negative Analyte
Seasonal Coronavirus	Parainfluenza Virus	1	Parainfluenza Virus
Human Rhinovirus/Enterovirus	Parainfluenza Virus	1	Parainfluenza Virus
Parainfluenza Virus	Respiratory Syncytial Virus	1	Respiratory Syncytial Virus
Adenovirus	Parainfluenza Virus	1	Adenovirus

2. Clinical Specificity:

Please refer to **Section C(1)**.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The rates of positive results for the SPOTFIRE R Panel analytes in the Prospective Clinical Study described in **Section VII (C)** are presented in **Tables 46** and **47** stratified by study site and age, respectively.

Table 46. Prevalence of analytes in prospectively collected nasopharyngeal swab specimens, as determined by the SPOTFIRE R Panel and comparator methods, stratified by site

Analyte	Test Method	Positive Results Stratified by Study Site (%)					
		All Sites (n =1120)	Site 1 (n = 361)	Site 2 (n = 36)	Site 3 (n = 511)	Site 4 (n = 147)	Site 5 (n = 65)
Adenovirus	SPOTFIRE	56 (5.0)	3 (0.8)	0	34 (6.7)	19 (12.9)	0
	Comparator	33 (3.0)	1 (0.3)	0	25 (4.9)	7 (4.8)	0
Seasonal Coronavirus	SPOTFIRE	114 (10.2)	24 (6.6)	4 (11.1)	73 (14.3)	13 (8.8)	0
	Comparator	102 (9.1)	19 (5.3)	3 (8.3)	67 (13.2)	13 (8.8)	0
SARS-CoV-2	SPOTFIRE	77 (6.9)	27 (7.5)	7 (19.4)	8 (1.6)	8 (5.4)	27 (41.5)
	Comparator	73 (6.6)	27 (7.5)	6 (16.7)	8 (1.6)	7 (4.8)	25 (39.1)
Human Metapneumovirus	SPOTFIRE	1 (0.1)	1 (0.3)	0	0	0	0
	Comparator	1 (0.1)	1 (0.3)	0	0	0	0
Human Rhinovirus/Enterovirus	SPOTFIRE	417 (37.2)	59 (16.3)	7 (19.4)	265 (51.9)	85 (57.8)	1 (1.5)
	Comparator	348 (31.2)	49 (13.6)	5 (13.9)	219 (43.1)	74 (50.3)	1 (1.6)
Influenza A Virus	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Influenza A Virus A/H1-2009	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Influenza A Virus A/H3	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Influenza B Virus	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Parainfluenza Virus	SPOTFIRE	107 (9.6)	20 (5.5)	3 (8.3)	72 (14.1)	11 (7.5)	1 (1.5)
	Comparator	98 (8.8)	16 (4.4)	3 (8.3)	68 (13.4)	11 (7.5)	0
Respiratory Syncytial Virus	SPOTFIRE	28 (2.5)	7 (1.9)	0	16 (3.1)	5 (3.4)	0
	Comparator	27 (2.4)	7 (1.9)	0	15 (3.0)	5 (3.4)	0
Bordetella parapertussis	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Bordetella pertussis	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Chlamydia pneumoniae	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Mycoplasma pneumoniae	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0

Samples with missing or invalid comparator results for specific analytes are excluded from the denominator in prevalence calculations for detection of those analytes using that method

Table 47. Prevalence of analytes in prospectively collected nasopharyngeal swab specimens, as determined by the SPOTFIRE R Panel and comparator methods, stratified by patient age

Analyte	Test Method	Positive Results Stratified by Age [years] (%)					
		All Ages (n =1120)	≤ 5 (n = 457)	6-18 (n = 258)	19-40 (n = 160)	41-60 (n = 147)	> 60 (n = 98)
Adenovirus	SPOTFIRE	56 (5.0)	53 (11.6)	3 (1.2)	0	0	0
	Comparator	33 (3.0)	31 (6.8)	2 (0.8)	0	0	0
Seasonal Coronavirus	SPOTFIRE	114 (10.2)	71 (15.5)	22 (8.5)	10 (6.3)	9 (6.1)	2 (2.0)
	Comparator	102 (9.1)	64 (14.1)	21 (8.2)	6 (3.8)	9 (6.2)	2 (2.0)
SARS-CoV-2	SPOTFIRE	77 (6.9)	9 (2.0)	7 (2.7)	27 (16.9)	21 (14.3)	13 (13.3)
	Comparator	73 (6.6)	9 (2.0)	5 (1.9)	25 (15.8)	20 (13.6)	14 (14.3)
Human Metapneumovirus	SPOTFIRE	1 (0.1)	1 (0.2)	0	0	0	0
	Comparator	1 (0.1)	1 (0.2)	0	0	0	0
Human Rhinovirus/Enterovirus	SPOTFIRE	417 (37.2)	236 (51.6)	139 (53.9)	23 (14.4)	14 (9.5)	5 (5.1)
	Comparator	348 (31.2)	200 (44.0)	116 (45.1)	17 (10.7)	11 (7.5)	4 (4.1)
Influenza A Virus	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Influenza A Virus A/H1-2009	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Influenza A Virus A/H3	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Influenza B	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Parainfluenza Virus	SPOTFIRE	107 (9.6)	89 (19.5)	4 (1.6)	4 (2.5)	8 (5.4)	2 (2.0)
	Comparator	98 (8.8)	83 (18.2)	4 (1.6)	3 (1.9)	6 (4.1)	2 (2.0)
Respiratory Syncytial Virus	SPOTFIRE	28 (2.5)	21 (4.6)	6 (2.3)	0	1 (0.7)	0
	Comparator	27 (2.4)	21 (4.6)	5 (1.9)	0	1 (0.7)	0
Bordetella parapertussis	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Bordetella pertussis	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Chlamydia pneumoniae	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0
Mycoplasma pneumoniae	SPOTFIRE	0	0	0	0	0	0
	Comparator	0	0	0	0	0	0

Samples with missing or invalid comparator results for specific analytes are excluded from the denominator in prevalence calculations for detection of those analytes using that method

F Other Supportive Instrument Performance Characteristics Data:

Not applicable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.