February 3, 2023

BioFire Diagnostics
Kevin Bourzac
Vice President, Regulatory & Clinical Affairs
515 Colorow Drive
Salt Lake City, Utah 84108

Re: K213954
Trade/Device Name: BIOFIRE SPOTFIRE Respiratory (R) Panel
Regulation Number: 21 CFR 866.3981
Regulation Name: Device to detect and identify nucleic acid targets in respiratory specimens from microbial agents that cause the SARS-CoV-2 respiratory infection and other microbial agents when in a multi-target test
Regulatory Class: Class II
Product Code: QOF, OEM, OOU, OTG, OZE, OZX, OZY, OZZ, OCC, NSU
Dated: December 16, 2021
Received: December 17, 2021

Dear Kevin Bourzac:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Joseph Briggs -S

Digitally signed by Joseph Briggs -S
Date: 2023.02.03 13:34:47 -05'00'

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

Enclosure

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K213954

Device Name
BIOFIRE® SPOTFIRE® Respiratory (R) Panel

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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BIOFIRE® SPOTFIRE® Respiratory (R) Panel

510(k) Summary
BioFire Diagnostics, LLC

Introduction:

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

Submitted by:

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

Telephone: 801-736-6354

Facsimile: 801-588-0507

Contact: Kevin Bourzac, Ph.D ext. 1358

Date Submitted: December 16, 2021

Device Name and Classification:

Trade Name: BIOFIRE® SPOTFIRE® Respiratory (R) Panel

Regulation Number: 21 CFR 866.3981

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

Predicate Device:

DEN200031 – BIOFIRE® Respiratory Panel 2.1 (RP2.1)

Intended Use:

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

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

Viruses	Bacteria
Adenovirus	*Bordetella parapertussis*
Coronavirus (seasonal)	*Bordetella pertussis*
Coronavirus SARS-CoV-2	*Chlamydia pneumoniae*
Human metapneumovirus	*Mycoplasma pneumoniae*
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	

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.

Device Description:

The BIOFIRE® SPOTFIRE® Respiratory (R) Panel (SPOTFIRE R Panel) simultaneously identifies 15 different respiratory viral and bacterial pathogens in nasopharyngeal swabs (NPS) from individuals with signs and symptoms of respiratory tract infection (see Table **1**) . The SPOTFIRE R Panel is compatible with the BIOFIRE® SPOTFIRE® System, a polymerase chain reaction (PCR)-based in vitro diagnostic system for infectious disease testing. The SPOTFIRE System Software executes the SPOTFIRE R Panel test and interprets and reports the test results. The SPOTFIRE R Panel was designed to be used in CLIA-waived environments.

Table 1. Analytes Detected by the SPOTFIRE (R) Panel

Viruses	Bacteria
Adenovirus	*Bordetella parapertussis*
Coronavirus (seasonal)	*Bordetella pertussis*
Coronavirus SARS-CoV-2	*Chlamydia pneumoniae*
Human metapneumovirus	*Mycoplasma pneumoniae*
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	

A test is initiated by loading Hydration Solution into one port of the SPOTFIRE R Panel pouch and a NPS specimen mixed with the provided Sample Buffer into the other port of the SPOTFIRE R Panel pouch and placing it in the SPOTFIRE System. The pouch contains all of the reagents required for specimen testing and analysis in a freeze-dried format; the addition of Hydration Solution and Sample/Buffer Mix rehydrates the reagents. After the pouch is prepared, the SPOTFIRE System Software guides the user through the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample identification, and initiating the run.

The SPOTFIRE System contains coordinated systems of inflatable bladders and seal points, which act on the pouch to control the movement of liquid between the pouch blisters. When a bladder is inflated over a reagent blister, it forces liquid from the blister into connecting channels. Alternatively, when a seal is placed over a connecting channel it acts as a valve to open or close a channel. In addition, electronically-controlled pneumatic pistons are positioned over multiple plungers in order to deliver the rehydrated reagents into the blisters at the appropriate times. Two Peltier devices control heating and cooling of the pouch to drive the PCR reactions and the melt curve analysis.

Nucleic acid extraction occurs within the SPOTFIRE R Panel pouch using mechanical and chemical lysis followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, the SPOTFIRE system performs a nested multiplex PCR that is executed in two stages. During the first stage, the SpotFire system performs a single, large volume, highly multiplexed reverse transcription PCR (rt-PCR) reaction. The products from first stage PCR are then diluted and combined with a fresh, primer-free master mix and a fluorescent double-stranded DNA binding dye (LC Green® *Plus*, BioFire Diagnostics). The solution is then distributed to each well of the array. Array wells contain sets of primers designed specifically to amplify sequences internal to the PCR products generated during the first stage PCR reaction. The 2nd stage PCR, or nested PCR, is performed in singleplex fashion in each well of the array. At the conclusion of the 2nd stage PCR, the array is interrogated by melt curve analysis for the detection of signature amplicons denoting the presence of specific targets. A digital camera placed in front of the 2nd stage PCR captures fluorescent images of the PCR reactions and software interprets the data.

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

Substantial Equivalence:

The SPOTFIRE R Panel is substantially equivalent to the BioFire Respiratory Panel 2.1 (BioFire RP2.1) (DEN200031), which was cleared on March 17, 2021 and determined to be a Class II device under the classification code 21 CFR 866.3981.

A table comparing the SPOTFIRE R Panel to the BioFire RP2.1 is provided in Table 2.

Table 2. Similarities and differences between the BioFire RP2.1 and the SPOTFIRE R Panel

Element	Predicate: BioFire RP2.1 (DEN200031)	New Device: SPOTFIRE R Panel
Intended Use	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 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 SPOTFIRE R Panel is a PCR-based multiplexed nucleic acid test intended for use with the SPOTFIRE System for the 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.
Specimen Types	Nasopharyngeal swab in transport media or saline	Nasopharyngeal swab in transport media
Organisms detected	**Viruses** Adenovirus Coronavirus 229E Coronavirus HKU1 Coronavirus NL63 Coronavirus OC43	Same, except: No assay for Influenza A subtype H1 Coronavirus 229E/HKU1/NL63/OC43 combined call (coronavirus seasonal)

	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Human Metapneumovirus Human Rhinovirus/Enterovirus Influenza A Subtypes: H1, H3 and 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*	Combined Parainfluenza call for serotypes 1-4
Analytes	DNA/RNA	Same
Technological Principles	Highly multiplexed nested nucleic acid amplification test with melt analysis	Same
Instrumentation	BioFire FilmArray 2.0 or BioFire FilmArray Torch Systems	SPOTFIRE System (this submission)
Time to result	About 1 hour	About 15 minutes
Reagent Storage	Room Temperature	Same
Test Interpretation	Automated test interpretation and reporting. User cannot access raw data.	Same
Controls	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis.	Same
User complexity	Moderate	Low (CLIA-waived)
Panel Software Functions	Defines panel-specific parameters, instrument protocols and report requirements.	Same
	Analyzes processed image data (fluorescence and temperature data) and provides test results.	Same

Summary of Performance Data

Clinical Performance

Prospective Clincal Evaluation

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

A total of 1215 NPS specimens were enrolled from consented volunteers or obtained as residual leftover specimens from subjects of all ages for the prospective clinical study; 95 of these NPS specimens were excluded. The most common reason for specimen exclusion was that a valid SPOTFIRE R Panel test was not obtained due to an invalid SPOTFIRE R Panel test. The final data set consisted of 1120 NPS specimens. Across the five study sites, 259 NPS specimens were initially collected and immediately frozen for later testing at the source study site. The remaining 861 NPS specimens were collected and tested fresh (without freezing). No difference in performance was observed when fresh and frozen specimen results were compared. Therefore, the data collected from 259 valid frozen specimens are combined with data from the valid 861 fresh specimens for all analyses.

Table 3 provides a summary of demographic information for the specimens included in the study.

Table 3. Demographic Summary for Prospective SPOTFIRE R Panel Clinical Evaluation

Category		Prospective NPS Specimens
Sex	Male	587 (52.4%)
	Female	533 (47.6%)
Age	≤5 years	457 (40.8%)
	6-18 years	258 (23.0%)
	19-40 years	160 (14.3%)
	41-60 years	147 (13.1%)
	61+ years	98 (8.8%)
Total		**1120**

The performance of the SPOTFIRE R Panel was evaluated by comparing the test results with those from FDA-cleared multiplexed respiratory pathogen panels. The performance for the prospective study is summarized in Table 4. Positive percent agreement (PPA) for each analyte was calculated as 100% × (TP / (TP + FN)). True positive (TP) indicates that both the SPOTFIRE R Panel and the comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the SPOTFIRE R Panel was negative while the comparator result was positive. Negative percent agreement (NPA) was calculated as 100% × (TN / TN + FP)). True negative (TN) indicates that both the SPOTFIRE R Panel and the comparator method had negative results, and false positive (FP) indicates that the SPOTFIRE R Panel was positive while the comparator method was negative. The exact binomial two-sided 95% confidence interval (95%CI) was calculated. Investigations of discrepant results are summarized in the footnotes.

Table 4. SPOTFIRE R Panel Clinical Performance Summary for NPS Specimens

SPOTFIRE R Panel Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Viruses						
Adenovirus[a]	32/33	97.0	84.7-99.5%	1058/1082	97.8	96.7-98.5%
Coronavirus SARS-CoV-2[b]	71/73	97.3	90.5-99.2%	1031/1037	99.4	98.7-99.7%
Coronavirus (seasonal)[c]	101/102	99.0	94.7-99.8%	1000/1013	98.7	97.8-99.2%
Human metapneumovirus	1/1	100	-	1114/1114	100	99.7-100%
Human rhinovirus/enterovirus[d]	345/348	99.1	97.5-99.7%	695/767	90.6	88.3-92.5%
Influenza A virus	0/0	-	-	1115/1115	100	99.7-100%
Influenza A virus A/H1-2009	0/0	-	-	1115/1115	100	99.7-100%
Influenza A virus A/H3	0/0	-	-	1115/1115	100	99.7-100%
Influenza B virus	0/0	-	-	1110/1110	100	99.7-100%
Parainfluenza virus[e]	96/98	98.0	92.9-99.4%	1006/1017	98.9	98.1-99.4%
Respiratory syncytial virus[f]	26/27	96.3	81.7-99.3%	1086/1088	99.8	99.3-99.9%
Bacteria						
Bordetella parapertussis	0/0	-	-	1110/1110	100	99.7-100%
Bordetella pertussis	0/0	-	-	1115/1115	100	99.7-100%
Chlamydia pneumoniae	0/0	-	-	1115/1115	100	99.7-100%
Mycoplasma pneumoniae	0/0	-	-	1115/1115	100	99.7-100%

[a] Adenovirus was not detected in the single FN specimen upon SPOTFIRE R Panel retest. Adenovirus was detected in 21/24 FP specimens using an additional molecular method.
[b] SARS-CoV-2 was detected in 1/2 FN specimens upon SPOTFIRE R Panel retest. SARS-CoV-2 was detected in 2/6 FP specimens using an additional molecular method.
[c] Seasonal coronavirus (229E/HKU1/NL63/OC43) was detected in the single FN specimen upon SPOTFIRE R Panel retest. Seasonal coronavirus (229E/HKU1/NL63/OC43) was detected in 8/13 FP specimens using an additional molecular method.
[d] Human rhinovirus/enterovirus was detected in 1/3 FN specimens upon SPOTFIRE R Panel retest. Human rhinovirus/enterovirus was detected in 48/72 FP specimens using an additional molecular method.
[e] Parainfluenza virus was detected in both FN specimens during discrepancy investigation upon SPOTFIRE R Panel retest. Parainfluenza virus was detected in 9/11 FP specimens using an additional molecular method.
[f] Respiratory syncytial virus was detected in the single FN specimen upon SPOTFIRE R Panel retest. Respiratory syncytial virus was detected in 1/2 FP specimens using an additional molecular method.

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

Testing of Preselected Archived Specimens

A number of analytes on the SPOTFIRE R Panel were of low prevalence during the prospective study and were not encountered in large enough numbers to adequately demonstrate system performance. To supplement the results of the prospective clinical study, an evaluation of preselected archived retrospective NPS specimens was performed.

A total of 562 frozen archived NPS specimens were obtained from 15 external laboratories world-wide and retrospectively tested at the four US clinical sites. Of these, 542 NPS specimens had valid results that were included in performance analysis. The analyte composition of the archived specimens was confirmed using the same comparator methods as the prospective study (described above) for the analyte result to be included in the performance analysis.

The specimens were randomized such that the users performing both the confirmation and the SPOTFIRE R Panel testing were blinded to the expected test result. A summary of the available demographic information of the tested specimens is provided in Table 5, and the results of the SPOTFIRE R Panel performance for these archived specimens is shown in Table 6.

Table 5. Demographic Summary for Valid Archived NPS Specimens

Category		Archived NPS Specimens
Sex	Male	254 (46.9%)
	Female	185 (34.1%)
	Unknown	103 (19.0%)
Age	≤5 years	234 (43.2%)
	6-18 years	98 (18.1%)
	19-40 years	36 (6.6%)
	41-60 years	35 (6.5%)
	61+ years	39 (7.2%)
	Unknown	100 (18.5%)
Total		**542**

Table 6. SPOTFIRE R Panel Archived Performance Summary for NPS Specimens

SPOTFIRE R Panel Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses						
Adenovirus[a]	31/31	100	89.0-100%	439/453	96.9	94.9-98.2%
Coronavirus SARS-CoV-2	0/0	-	-	0/0	-	-
Coronavirus (seasonal)[b]	95/96	99.0	94.3-99.8%	381/388	98.2	96.3-99.1%
Human metapneumovirus[c]	32/33	97.0	84.7-99.5%	451/451	100	99.2-100%
Human rhinovirus/enterovirus[d]	29/30	96.7	83.3-99.4%	439/454	96.7	94.6-98.0%
Influenza A virus[e]	58/59	98.3	91.0-99.7%	423/423	100	99.1-100%
Influenza A virus A/H1-2009[e]	31/32	96.9	84.3-99.4%	450/450	100	99.2-100%
Influenza A virus A/H3	27/27	100	87.5-100%	455/455	100	99.2-100%
Influenza B virus	30/30	100	88.6-100%	28/28	100	87.9-100%
Parainfluenza virus[f]	116/118	98.3	94.0-99.5%	359/366	98.1	96.1-99.1%
Respiratory syncytial virus[g]	37/37	100	90.6-100%	440/447	98.4	96.8-99.2%
Bacteria						
Bordetella parapertussis[h]	24/25	96.0	80.5-99.3%	33/33	100	89.6-100%
Bordetella pertussis[i]	27/28	96.4	82.3-99.4%	452/456	99.1	97.8-99.7%
Chlamydia pneumoniae[j]	30/30	100	88.6-100%	452/454	99.6	98.4-99.9%
Mycoplasma pneumoniae[k]	33/33	100	89.6-100%	446/451	98.9	97.4-99.5%

[a] Adenovirus was detected in 6/12 FP specimens during discrepancy investigation: one was detected by standard of care and five were detected using an additional molecular method; two additional FP specimens were unable to be investigated.

[b] The single FN specimen was unable to be investigated. Seasonal coronavirus (229E/HKU1/NL63/OC43) was detected in 3/6 FP specimens during discrepancy investigation using an additional molecular method; one additional FP specimen was unable to be investigated.

[c] Human metapneumovirus was detected in the single FN specimen by standard of care.

[d] The single FN specimen was unable to be investigated. Human rhinovirus/enterovirus was detected in 4/14 FP specimens during discrepancy investigation using an additional molecular method; one additional FP specimen was unable to be investigated.

[e] Influenza A virus A/H1-2009 was detected in the single FN specimen by standard of care.

[f] Parainfluenza virus was detected in the single FN specimen by standard of care; one additional FN specimen was unable to be investigated. Parainfluenza virus was detected in all seven FP specimens during discrepancy investigation: six were detected by standard of care and one was detected using an additional molecular method.

[g] Respiratory syncytial virus was detected in 4/6 FP specimens during discrepancy investigation using an additional molecular method; one additional FP specimen was unable to be investigated.

[h] *Bordetella parapertussis* was detected in the single FN specimen by standard of care.

[i] *Bordetella pertussis* was detected in the single FN specimen by standard of care. *Bordetella pertussis* was detected in 3/4 FP specimens by standard of care.

[j] *Chlamydia pneumoniae* was detected in both FP specimens by standard of care.

[k] *Mycoplasma pneumoniae* was detected in all five FP specimens during discrepancy investigation: four were detected by standard of care and one was detected using an additional molecular method.

Analytical Performance Characteristics

Limit of Detection

The limit of detection (LoD) for SPOTFIRE R Panel analytes was estimated by testing dilutions of contrived samples containing known concentrations of organism in artificial nasopharyngeal swab matrix in VTM. The LoD concentrations were confirmed by testing at least 20 replicates at the estimated LoD. Confirmation of LoD required detection in at least 95% of replicates tested. The confirmed LoD concentrations for the SPOTFIRE R Panel are listed in Table 7.

Table 7. Limit of Detection (LoD) for the SPOTFIRE R Panel Analytes

Analyte	Isolate Source ID	LoD Concentration[a]
Viruses		
Adenovirus	Species A Serotype 31 ZeptoMetrix 0810073CF	4.1E-03 $TCID_{50}$/mL (1.0E+02 copies/mL)
	Species B Serotype 3 ZeptoMetrix 0810062CF	8.0E-01 $TCID_{50}$/mL (8.4E+02 copies/mL)
	Species C Serotype 2 WHO I.S. NIBSC 16-324	8.2E+02 IU/mL[b] (8.2E+02 copies/mL[b])
	Species D Serotype 37 ZeptoMetrix 0810119CF	1.1E-02 $TCID_{50}$/mL (4.5E+02 copies/mL)
	Species E Serotype 4 ZeptoMetrix 0810070CF	1.8E-02 $TCID_{50}$/mL (1.0E+04 copies/mL)
	Species F Serotype 41 ZeptoMetrix 0810085CF	1.4E-02 $TCID_{50}$/mL (1.0E+02 copies/mL)
Coronavirus (seasonal)	229E ATCC VR-740	6.5E-01 $TCID_{50}$/mL (1.1E+01 copies/mL)
	HKU1 Clinical Specimen[c]	1.8E+04 copies/mL
	OC43 ZeptoMetrix 0810024CF	1.6E-02 $TCID_{50}$/mL (6.3E+01 copies/mL)
	NL63 ZeptoMetrix 0810228CF	2.5E-03 $TCID_{50}$/mL (4.7E+00 copies/mL)
Coronavirus SARS-CoV-2	USA-WA1/2020 (heat inactivated) ATCC VR-1986HK	1.1E-01 $TCID_{50}$/mL (2.5E+02 copies/mL)
Human metapneumovirus	A1-16 Iowa 10/2003 ZeptoMetrix 0810161CF	3.2E+00 $TCID_{50}$/mL (2.4E+02 copies/mL)
	B1-3 Peru2-2002 ZeptoMetrix 0810156CF	2.5E-01 $TCID_{50}$/mL (5.4E+02 copies/mL)
	A2-27 Iowa A/2004 ZeptoMetrix 0810164CF	5.8E-01 $TCID_{50}$/mL (1.8E+03 copies/mL)
	B2-18 IA18-2003 ZeptoMetrix 0810162CF	2.0E+00 $TCID_{50}$/mL (7.7E+02 copies/mL)
Human rhinovirus/enterovirus	Human Rhinovirus 1A ZeptoMetrix 0810012CFN	2.1E-01 $TCID_{50}$/mL (1.1E+00 copies/mL)
	Enterovirus D68 US/MO/14-18947 ATCC VR-1823	1.1E+01 $TCID_{50}$/mL (5.4E+01 copies/mL)
Influenza A virus Subtype H1-2009	Influenza A H1N1 pdm A/Michigan/45/15 ZeptoMetrix 0810538CF	8.2E-01 $TCID_{50}$/mL (3.4E+02 copies/mL
Influenza A virus Subtype H3	Influenza A H3N2 A/Hong Kong/4801/14 ZeptoMetrix 0810526CF	8.6E-01 $TCID_{50}$/mL (3.4E+02 copies/mL)
Influenza B virus	B/Florida/02/06 (Victoria Lineage) ZeptoMetrix 0810037CF	3.3E-02 $TCID_{50}$/mL (1.6E+02 copies/mL)
	B/Nevada/03/2011 (Victoria Lineage) BEI NR-44023	1.6E+00 $CEID_{50}$/mL (4.3E+00 copies/mL)
	B/Florida/04/06 (Yamagata Lineage) ZeptoMetrix 0810255CF	4.0E-01 $TCID_{50}$/mL (3.2E+01 copies/mL)
Parainfluenza virus	Serotype 1 ZeptoMetrix 0810014CF	4.6E+00 $TCID_{50}$/mL (1.4E+03 copies/mL)
	Serotype 2 ZeptoMetrix 0810015CF	1.4E+01 $TCID_{50}$/mL (1.6E+02 copies/mL)
	Serotype 3 ZeptoMetrix 0810016CF	2.6E+01 $TCID_{50}$/mL (6.1E+01 copies/mL)

Analyte	Isolate Source ID	LoD Concentration[a]
	Serotype 4 ZeptoMetrix 0810060CF	2.0E+02 $TCID_{50}$/mL (1.1E+03 copies/mL)
Respiratory syncytial virus	Type A 2006 ZeptoMetrix 0810040ACF	6.2E-02 $TCID_{50}$/mL (2.2E+01 copies/mL)
	Type B 3/2015 Isolate #1 ZeptoMetrix 0810479CF	2.8E-02 $TCID_{50}$/mL (2.4E+01 copies/mL)
Bacteria		
Chlamydia pneumoniae	AR-39 ATCC 53592	2.0E+01 IFU/mL (1.4E+02 copies/mL)
Mycoplasma pneumoniae	M129 ZeptoMetrix 0801579	1.0E+01 CCU/mL (2.1E+03 copies/mL)
Bordetella parapertussis	E595 ZeptoMetrix 0801462	4.0E+01 CFU/mL
Bordetella pertussis	A639 ZeptoMetrix 0801459	3.3E+02 CFU/mL (3.8E+02 copies/mL)

[a] LoD concentration may vary from what is listed based on the accuracy and precision of the quantification method.
[b] IU = International Units; BioFire Diagnostics quantified the WHO International Standard by quantitative real-time PCR to demonstrate that 8.2E+02 IU/mL=8.2E+02 copies/mL
[c] Testing for Coronavirus HKU1 utilized a clinical specimen due to the lack of availability of a cultured isolate. Viral concentration was determined in RNA copies/mL by quantitative real-time RT-PCR.

NOTE: LoD concentrations in copies/mL in *Error! Reference source not found.* ***above are based on extraction of nucleic acids from isolate cultures followed by quantitative real-time PCR (qPCR) or digital PCR (dPCR). The accuracy of concentrations may be affected by extraction efficiency, standard curve accuracy (qPCR only), assay conditions, inhibitors, and/or sequence variance. The quantification has not been compared to a reference material or other quantification methods.***

NOTE: LoD concentrations of cultured viruses provided in units of $TCID_{50}$ (50% Tissue Culture Infectious Dose) or $CEID_{50}$ (50% Chicken Embryo Infectious Dose) are not a direct count of viral particles or nucleic acid, but an indirect measure of viral concentration based on infectivity and cytotoxicity. $TCID_{50}$/mL and $CEID_{50}$/mL will therefore vary depending on technique and methodology (including cell type, culture media and conditions, cytotoxicity of the virus, etc.). It is not appropriate to make determinations on relative sensitivity of detection for different cultures and/or different molecular assays based on LoD values measured in $TCID_{50}$/mL or $CEID_{50}$/mL.

Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the SPOTFIRE R Panel assays was assessed by testing viral and bacterial isolates that represented clinical and genetic diversity and included the available phylogenetic, geographic, and temporal diversity of each SPOTFIRE R Panel analyte. Isolates were tested in triplicate at concentrations near the LoD.

A summary of analytical reactivity is included in Table 8 to Table 19.

NOTE: Influenza A assays will react variably with non-human influenza A viruses and rarely encountered human influenza A viruses that are not H1-2009 or H3; generally producing Uncertain: Influenza A virus or Influenza A virus (No Subtype Identified) results.

NOTE: The SPOTFIRE R Panel assays may react with vaccines that contain specific segments of the pathogen genome or full genome or vaccines containing attenuated/inactivated pathogen, including vaccines for SARS-CoV-2, influenza A (various subtypes), influenza B, poliovirus (human rhinovirus/enterovirus), and Bordetella pertussis. Care should be taken to minimize contamination of samples with vaccines, and clinical history of vaccine administration should be considered in the interpretation of results, particularly for vaccines administered by nasal spray.

Table 8. Summary of Reactivity to Adenovirus Isolates

Species	Type	Source/Isolate ID	[Strain/Location/Year]	Result
A	12	ATCC VR-863	[Huie/Massachusetts]	**Adenovirus Positive**
	18	ATCC VR-19	[Washington DC/1954]	
	31	ZeptoMetrix 0810073CF	-	
B	3	ZeptoMetrix 0810062CF	-	
	7	ATCC VR-7	[Gomen/California/1954]	
	7a	ZeptoMetrix 0810021CF	-	
	7d/d2	UIRF	[Iowa/2001]	
	11	ATCC VR-12	[Slobitski/Massachusetts]	
	14	ATCC VR-15	[De Wit/Netherlands/1955]	
	16	ATCC VR-17	[CH.79/Saudi Arabia/1955]	
	21	ATCC VR-1833	[128/Saudi Arabia/1956]	
	34	ATCC VR-716	[Compton/1972]	
	35	ATCC VR-718	[Holden]	
	50	ATCC VR-1602	[Wan, RIVM no. 88-1773]	
C	1	ZeptoMetrix 0810050CF	-	
	2	W.H.O NIBSC 16/324	-	
	5	ZeptoMetrix 0810020CF	-	
	6	ATCC VR-6	[Tonsil 99]	
D	8	ZeptoMetrix 0810069CF	-	
	20	ZeptoMetrix 0810115CF	[KB]	
	37	ZeptoMetrix 0810119CF	-	
E	4	ZeptoMetrix 0810070CF	-	
	4	ATCC VR-1572	[RI-67/Missouri/1952-1953]	
	4a	UIRF	[S.Carolina/2004]	
F	40	NCPV 0101141v	-	
	40	ZeptoMetrix 0810084CF	-	
	41	ATCC VR-930	[Tak (73-3544)]	
	41	ZeptoMetrix 0810085CF	[Tak]	

Table 9. Summary of Reactivity to *Bordetella parapertussis* Isolates

Source/Isolate ID	Strain/Location/Year	Result
ZeptoMetrix 0801462	[E595]	***Bordetella parapertussis* Positive**
ATCC 9305	[517]	
ATCC 53892	[PT28G]	
ATCC 53893	[PT 26/28G]	
ATCC 15237	[NCTC 10853]	
ATCC 15311	[NCTC 5952]	
ATCC BAA-587	[12822/Germany/1993]	
ZeptoMetrix 0801461	[A747]	
ZeptoMetrix 0801643	[C510]	
ZeptoMetrix 0801644	[E838]	

Table 10. Summary of Reactivity to *Bordetella pertussis* Isolates

Source/Isolate ID	Strain/Location/Year	Result
ZeptoMetrix 0801459	[A639]	***Bordetella pertussis* Positive**
ATCC 10380	[10-536]	
ATCC 51445	[CNCTC Hp 12/63 [623]]	
ATCC 8467	[F]	
ATCC 9340	[5 [17921]	
ATCC 9797	[18323 [NCTC 10739]	
ATCC BAA-1335	[MN2531]	
ATCC BAA-589	[Tohama]	
ZeptoMetrix 0801460	[E431]	

Table 11. Summary of Reactivity to *Chlamydia pneumoniae* Isolates

Source/Isolate ID	Strain/Location/Year	Result
ATCC 53592	[AR-39]	***Chlamydia pneumoniae* Positive**
ATCC VR-1310	[CWL-029]	
ATCC VR-1356	[TWAR strain 2023]	
ATCC VR-1360	[CM-1]	
ATCC VR-1435	[J-21]	
ATCC VR-1452	[A03]	
ATCC VR-2282	[TWAR strain, TW-183/Taiwan/1965]	

Table 12. Summary of Reactivity to Coronavirus Isolates

Type	Source/Isolate ID	Strain/Location/Year	Result
NL63	ZeptoMetrix 0810228CF	-	**Coronavirus (seasonal)**

Type	Source/Isolate ID	Strain/Location/Year	Result
	BEI NR-470	[Amsterdam/2003]	**Positive**
229E	ATCC VR-740	-	
	ZeptoMetrix 0810229CF	-	
HKU1	Clinical Specimen	[Columbus OH, 2016]	
	Clinical Specimen	[South Carolina/2010]	
	Clinical Specimen	[France/2016]	
	Clinical Specimen	[France/2016]	
OC43	ZeptoMetrix 0810024CF	-	
	ATCC VR-759	-	
SARS-CoV-2 [a]	ATCC VR-1986HK	[USA-WA1/2020]	**Coronavirus SARS-CoV-2 Positive**
	ATCC VR-1991D	[Hong Kong/VM20001061/2020]	
	ATCC VR-1992D	[2019-nCoV/Italy-INMI1]	
	ATCC VR-1994D	[Germany/BavPat1/2020]	
	ATCC VR-3326D	[USA/CA_CDC_5574/2020]	
	BEI NR-52499 [b]	[England/02/2020]	
	BEI NR-52501 [c]	[Singapore/2/2020]	
	BEI NR-52503 [d]	[USA-IL1/2020]	
	BEI NR-52505 [e]	[USA-AZ1/2020]	
	BEI NR-52507 [f]	[USA-CA3/2020]	
	BEI NR-52510 [g]	[Chile/Santiago_op4d1/2020]	
	BEI NR-53518 [h]	[New York-PV08410/2020]	
	LGC SeraCare AccuPlex™ 0505-0298[i]	[Omicron B.1.1.529 Variant]	

[a] See **Error! Reference source not found.** for additional SARS-CoV-2 reactivity predictions based on *in silico* analysis.
[b] The following reagent was deposited by Professor Maria Zambon and obtained through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate England/02/2020, NR-52499.
[c] The following reagent was contributed by Duke-National University of Singapore, Programme in Emerging Infectious Diseases for distribution through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate Singapore/2/2020, NR-52501.
[d] The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-IL1/2020, NR-52503.
[e] The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-AZ1/2020, NR-52505.
[f] The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-CA3/2020, NR-52507.
[g] The following reagent was obtained through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate Chile/Santiago_op4d1/2020, NR-52510.
[h] The following reagent was obtained through BEI Resources, NIAID, NIH: Genomic RNA from SARS-Related Coronavirus 2, Isolate New York-PV08410/2020, NR-53518.
[i] Recombinant alphavirus that contains the full SARS-CoV-2 genome with mutations identified in the S and N gene of the SARS-CoV-2 Omicron variant B.1.1.529.

Table 13. Summary of Reactivity to Human Rhinovirus and Enterovirus Isolates

Species	Serotype	Source/Isolate ID	[Strain/Location/Year]	Result
Human Rhinovirus				
A	1	ZeptoMetrix 0810012CFN	[1A]	**Human rhinovirus/enterovirus Positive**
	77	ATCC VR-1187	[130-63]	
	85	ATCC VR-1195	[50-525-CV54]	
	34	ATCC VR-1365	[137-3]	
	57	ATCC VR-1600	[Ch47]	
	7	ATCC VR-1601	[68-CV11]	
	16	ATCC VR-283	[11757]	
	2	ATCC VR-482	[HGP]	
B	17	ATCC VR-1663	[33342]	
	14	ATCC VR-284	[1059]	
	42	ATCC VR-1950	[56822]	
	3	ATCC VR-483	[FEB]	
	27	ATCC VR-1137	[5870]	
	83	ATCC VR-1193	[Baylor 7]	
Enterovirus				
A	Enterovirus 71	ATCC VR-1432	[71 H]	**Human rhinovirus/enterovirus Positive**
	Coxsackievirus 10	ATCC VR-168	[NY/1950]	
B	Coxsackievirus 9	ZeptoMetrix 0810017CF	-	
	Echovirus 11	ZeptoMetrix 0810023CF	-	
	Coxsackievirus B3	ZeptoMetrix 0810074CF	-	
	Coxsackievirus B4	ZeptoMetrix 0810075CF	-	
	Echovirus 6	ZeptoMetrix 0810076CF	-	
	Echovirus 9	ZeptoMetrix 0810077CF	-	
C	Coxsackievirus A24	ATCC VR-583	[DN-19/TX/1963]	
	Coxsackievirus A21	ATCC VR-850	[Kuykendall/CA/1952]	
D	Enterovirus D68	ATCC VR-1823	[US/MO/14-18947]	

Table 14. Summary of Reactivity to Human Metapneumovirus Isolates

Genotype	Serotype	Source/Isolate ID	[Location/Year]	Result
A1	9	ZeptoMetrix 0810160CF	[Iowa 3/2002]	**Human metapneumovirus Positive**
	16	ZeptoMetrix 0810161CF	[Iowa 10/2003]	
A2	20	ZeptoMetrix 0810163CF	[Iowa 14/2003]	
	27	ZeptoMetrix 0810164CF	[Iowa 27/2004]	
B1	3	ZeptoMetrix 0810156CF	[Peru2-2002]	
	5	ZeptoMetrix 0810158CF	[Peru 3/2003]	
B2	4	ZeptoMetrix 0810157CF	[Peru 1/2002]	
	8	ZeptoMetrix 0810159CF	[Peru 6/2003]	
	18	ZeptoMetrix 0810162CF	[IA18-2003]	
	Unknown	BEI NR-22232	[TN/91-316]	

Table 15. Summary of Reactivity to Influenza A Isolates

Type	Host	Source/Isolate ID	Strain/Location/Year	Result
H1N1pdm09	Human	ZeptoMetrix 0810538CF	[Michigan/45/15]	**Influenza A virus (Subtype H1-2009) Positive**
		BEI NR-19823	[Netherlands/2629/2009]	
		BEI NR-42938	[Georgia/F32551/2012]	
		BEI NR-44345	[Hong Kong/H090-761-V1(0)/2009]	
		ZeptoMetrix 0810109CFJ	[Canada/6294/2009]	
		ZeptoMetrix 0810165CF	[California/07/2009]	
		ZeptoMetrix 0810166CF	[Mexico/4108/2009]	
		ZeptoMetrix 0810249CF	[SwineNY/03/2009]	
H3N2	Human	ATCC VR-544	[Hong Kong/8/1968]	**Influenza A virus (Subtype H3) Positive**
		ATCC VR-547	[Aichi/2/1968]	
		ATCC VR-776	[Alice]	
		ATCC VR-810	[Port Chalmers/1/1973]	
		ATCC VR-822	[Victoria/3/1975]	
		ZeptoMetrix 0810138CF	[Brisbane/10/2007]	
		ZeptoMetrix 0810238CF	[Texas/50/2012]	
		ZeptoMetrix 0810252CF	[Wisconsin/67/2005]	
		ZeptoMetrix 0810526CF	[Hong Kong/4801/14]	
H1N1	Human	ZeptoMetrix 0810036CF	[New Caledonia/20/1999]	**Influenza A virus Positive (No Subtype Identified)**
		ZeptoMetrix 0810036CFN	[Solomon Islands/3/2006]	
		ZeptoMetrix 0810244CF	[Brisbane/59/2007]	
	Swine	ATCC VR-333	[Swine/Iowa/15/1930]	
		ATCC VR-897	[Swine/A/New Jersey/8/76]	
		ATCC VR-99	[Swine/1976/1931]	
H2N2	Human	BEI NR-2775 [a]	[A/Japan/305/1957]	
H5N3	Avian	BEI NR-9682 [b]	[A/Duck/Singapore/645/97]	
H1N2	Recombinant	BEI NR-3478 [c]	[Kilbourne F63 A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA)]	**Uncertain: Influenza A virus**
H10N7	Avian	BEI NR-2765 [d]	[A/Chicken/Germany/N/49]	

[a] The following reagent was obtained through BEI Resources, NIAID, NIH: Genomic RNA from Influenza A Virus, A/Japan/305/1957 (H2N2), NR-2775.
[b] The following reagent was obtained through BEI Resources, NIAID, NIH: Genomic RNA from Kilbourne F181: A/duck/Singapore/645/1997 (H5N3), Wild Type, NR-9682.
[c] The following reagent was obtained through BEI Resources, NIAID, NIH: Kilbourne F63: A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA) (H1N2), Reassortant NWS-F, NR-3478.
[d] The following reagent was obtained through BEI Resources, NIAID, NIH: Genomic RNA from Influenza A Virus, A/chicken/Germany/N/1949 (H10N7), NR-2765.

Table 16. Summary of Reactivity to Influenza B Isolates

Lineage	Source/Isolate ID	[Strain/Location/Year]	Result
Yamagata	ZeptoMetrix 0810255CF	[Florida/04/06]	Influenza B virus Positive
	ZeptoMetrix 0810239CF	[2/Massachusetts/2012]	
	ZeptoMetrix 0810241CF	[1/Wisconsin/2010]	
	ZeptoMetrix 0810256CF	[07/Florida/2004]	
Victoria	ZeptoMetrix 0810037CF	[B/Florida/02/06]	
	BEI NR-44023	[B/Nevada/03/2011]	
	ATCC VR-823	[5/Hong Kong/1972]	
	CDC 2005743348	[1/Ohio/2005]	
	ZeptoMetrix 0810258CF	[2506/Malaysia/2004]	
Unknown	ATCC VR-101	[Lee/1940]	
	ATCC VR-102	[Allen/1945]	
	ATCC VR-103	[GL/1739/1954]	
	ATCC VR-295	[2/Taiwan/1962]	
	ATCC VR-296	[1/Maryland/1959]	
	ATCC VR-786	[Brigit/Russia/1969]	

Table 17. Summary of Reactivity to *Mycoplasma pneumoniae* Isolates

Source/Isolate ID	Strain/Location/Year	Result
ZeptoMetrix 0801579	[M129]	

Source/Isolate ID	Strain/Location/Year	Result
ATCC 29085	[PI 1428]	*Mycoplasma pneumoniae* Positive
ATCC 29342	[M129-B7]	
ATCC 15492	[Mac]	
ATCC 15531-TTR	[FH strain of Eaton Agent [NCTC 10119]	
ATCC 15293	[M52]	
ATCC 15377	[Bru]	
ATCC 39505	[Mutant 22]	
ATCC 49894	[UTMP-10P]	

Table 18. Summary of Reactivity to Parainfluenza Virus Isolates

Serotype	Subtype	Source/Isolate ID	Strain/Location/Year	Result
1		ZeptoMetrix 0810014CF	-	**Parainfluenza virus Positive**
		ATCC VR-94	[C-35/1957]	
		BEI NR-48680	[FRA/29221106/2009]	
2		ZeptoMetrix 0810015CF	-	
		ATCC VR-92	[Greer/1955]	
3		ZeptoMetrix 0810016CF	-	
		ATCC VR-93	[C-243/1957]	
		BEI NR-3233	[NIH 47885 Wash/47885/57]	
4	A	ZeptoMetrix 0810060CF	-	
		ATCC VR-1378	[M-25/1958]	
		ZeptoMetrix 0810060CF	-	
	B	ATCC VR-1377	[CH-19503/1962]	
		ZeptoMetrix 0810060BCF	-	

Table 19. Summary of Reactivity to Respiratory Syncytial Virus Isolates

Type	Source/Isolate ID	Strain/Location/Year	Result
A	ZeptoMetrix 0810040ACF	[2006]	**Respiratory syncytial virus Positive**
	ATCC VR-26	[Long/Maryland/1956]	
	ATCC VR-1540	[A2/Melbourne/1961]	
	ZeptoMetrix 0810474CF	[2/2015 Isolate #2]	
	ZeptoMetrix 0810452CF	[12/2014 Isolate #2]	
B	ZeptoMetrix 0810479CF	[3/2015 Isolate #1]	
	ZeptoMetrix 0810040CF	[Ch-93 (18)-18]	
	ATCC VR-1400	[WV/14617/1985]	
	ATCC VR-955	[9320/Massachusetts/1977]	
	ATCC VR-1580	[18537/WashingtonDC/1962]	
	ZeptoMetrix 0810451CF	[11/2014 Isolate #2]	

In Silico Reactivity Predictions for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Assays

Evaluation of analytical reactivity for the SPOTFIRE R Panel SARS-CoV-2 assays (SARS-CoV2-1 and SARS-CoV2-2) was based on *in silico* analysis of all available sequences in the GISAID database as of December 21, 2022. In total, 11,989,970 sequences from around the globe were aligned to the assay primers.

This analysis determined that the >99.99% of 11,079,180 sequences will be detected by one or both SPOTFIRE R Panel SARS-CoV-2 assays based on homology and mismatch location with one or both sets of primers. A limitation on detection (both assays impaired) is predicted for less than 0.004% of the sequences evaluated (427 / 11,989,970) (Table 20).

The sequences evaluated include lineages and variants of concern (VOC) or variants under investigation (VUI) that may have important epidemiological, immunological, or pathogenic properties from a public health perspective, such as Delta and Omicron variants. Variants evaluated are listed in *the BioFire® Respiratory Panels SARS-CoV-2 Reactivity Tech Note* technical note at www.biofiredx.com/support/documents.

All lineages and variants of public health interest identified as of December 2022 are predicted to be detected; new sequences and variants will continue to be monitored for impacts on detection by the SPOTFIRE R Panel assays.

Table 20. *In silico* Prediction of SARS-CoV-2 Detection by SPOTFIRE R Panel Assays

+/+ indicates detected by both assays with no impairment, +/- indicates detection by one assay with no impairment and potential for impaired detection by the other assay, -/- indicates potential for impaired detection by both assays

***In silico* prediction**				
Predicted Assay Result		**SARS-CoV2-1**		**Total Sequences**
Number of Sequences		**+**	**-**	
SARS-CoV2-2	**+**	11,759,674	185,745	11,989,543 / 11,989,970
	-	44,124	427	99.9964%

Analytical Specificity (Cross-Reactivity & Exclusivity)

The potential for non-specific amplification and detection by the SPOTFIRE R Panel assays was evaluated by in silico analysis of available sequences and also by testing of high concentrations of on-panel and off-panel organisms. The organisms evaluated included relevant bacteria, fungi, and viruses that are either phylogenetically related to organisms detected by the SPOTFIRE R Panel or pathogenic/commensal organisms that may be present in NPS specimens. Each organism was tested in triplicate at the highest possible concentration (generally ≥1.0E+07 units/mL for bacteria and ≥1.0E+05 units/mL for viruses).

In silico analysis and testing identified a risk of SARS-CoV-2 assay cross-reactivity with a few sequences of SARS-like viruses isolated from bats and pangolin as well as intra-panel cross-reactivity with Bordetella species and Influenza A subtypes of swine origin. A summary of potential cross-reactivity is provided in Table 21. The on-panel and off-panel isolates and concentrations tested are listed in Table 22 and Table 23 respectively.

Table 21. Predicted and Observed Cross-Reactivity of the SPOTFIRE R Panel

Cross-Reactive Organism/Sequence	SPOTFIRE R Panel Analyte Result	Description
Bat coronavirus **Pangolin coronavirus** **Bat SARS-like coronavirus**	**Coronavirus SARS-CoV-2**[a]	The SARS-CoV-2 assays can amplify select sequences from closely related sarbecoviruses isolated from bats and pangolin.
Bordetella bronchiseptica (isolates containing IS*1001* gene)	***Bordetella parapertussis***	Some strains of *B. bronchiseptica* contain IS*1001* insertion sequences identical to those present in *B. parapertussis*. These sequences will be amplified by the IS1001 assay and reported as *Bordetella parapertussis*.
Bordetella bronchiseptica[b] ***Bordetella parapertussis***[b] ***Bordetella pertussis***	**Human rhinovirus/enterovirus**[c]	The HRV/EV assay designed to target Human Rhinovirus/Enterovirus may amplify the oxidoreductase gene from *Bordetella* species (*B. pertussis, B. parapertussis,* and *B. bronchiseptica*) when organisms are present at a high concentration. Cross-reactivity with *B. pertussis* was observed at a concentration of ≥1.3E+10 CFU/mL.
Bovine picornavirus **Canine picornavirus**	**Human rhinovirus/enterovirus**[a]	Bovine and canine picornaviruses may be detected and reported as Human rhinovirus/enterovirus when present at high concentration.
Influenza A H1N1 (swine origin)	**Influenza A Subtype H1-2009**[a]	The FluA-H1-2009 assay may react with the H1 hemagglutinin gene sequences from viruses of swine origin. The SPOTFIRE R Panel will report either Influenza A Virus (Subtype H1-2009) or Influenza A Virus (No Subtype Identified), depending on the strain and concentration in the sample.
Chlamydia gallinacea	***Chlamydia pneumoniae***[a]	*Chlamydia gallinacea* may be detected and reported as *Chlamydia pneumoniae* when present at high concentration.

[a] Indicated cross-reactivity is predicted based on *in silico* analysis.
[b] Cross-reactivity between the HRV/EV assay and *B. bronchiseptica* and *B. parapertussis* is predicted based on *in silico* analysis but was not observed when testing organisms at the highest possible concentrations (8.3E+09 cells/mL for *B. bronchiseptica* and 4.6E+09 CFU/mL for *B. parapertussis*).
[c] Cross-reactivity between the HRV/EV assay and *B. parapertussis* or *B. pertussis* will have positive results reported for Human Rhinovirus/Enterovirus and *B. parapertussis* or *B. pertussis*, respectively. Cross-reactivity between the HRV/EV assay and *B. bronchiseptica* isolates that do not carry the IS*1001* sequence will only have positive results reported for Human rhinovirus/enterovirus. Cross-reactivity between the HRV/EV assay and *B. bronchiseptica* isolates that contain the IS*1001* sequence will have positive results reported for Human Rhinovirus/Enterovirus and *B. parapertussis*.

Table 22. Summary of Results for On-Panel Organisms Tested During Evaluation of Analytical Specificity of the SPOTFIRE R Panel

Organism	Isolate ID	Concentration Tested	Observed Cross-Reactivity
Bacteria			
Bordetella parapertussis	ZeptoMetrix 0801462	4.6E+09 CFU/mL	None
Bordetella pertussis	ZeptoMetrix 0801459	1.3E+10 CFU/mL	**Human rhinovirus/enterovirus**[a]
		1.3E+09 CFU/mL	None
Chlamydia pneumoniae	ATCC 53592	2.9E+07 IFU/mL	None
Mycoplasma pneumoniae	ZeptoMetrix 0801579	2.5E+07 CCU/mL	None
Viruses			
Adenovirus A	ZeptoMetrix 0810073CF	1.4E+05 $TCID_{50}$/mL	None
Adenovirus B	ZeptoMetrix 0810062CF	1.2E+07 $TCID_{50}$/mL	None
Adenovirus C	ZeptoMetrix 0810110CF	2.2E+06 $TCID_{50}$/mL	None
Adenovirus D	ZeptoMetrix 0810119CF	1.7E+05 $TCID_{50}$/mL	None
Adenovirus E	ZeptoMetrix 0810070CF	1.4E+05 $TCID_{50}$/mL	None
Adenovirus F	ZeptoMetrix 0810085CF	1.1E+06 $TCID_{50}$/mL	None
Coronavirus 229E	ATCC VR-740	8.9E+06 $TCID_{50}$/mL	None
Coronavirus HKU1	Clinical Specimens	4.5E+07 copies/mL	None
Coronavirus NL63	ZeptoMetrix 0810228CF	5.0E+05 $TCID_{50}$/mL	None
Coronavirus OC43	ZeptoMetrix 0810024CF	3.6E+05 $TCID_{50}$/mL	None
Coronavirus SARS-CoV-2 (heat-inactivated)	ATCC VR-1986HK	7.6E+07 copies/mL	None
Enterovirus D68	ATCC VR-1823	1.6E+07 $TCID_{50}$/mL	None
Human Metapneumovirus A1	ZeptoMetrix 0810161CF	2.5E+05 $TCID_{50}$/mL	None
Human Metapneumovirus A2	ZeptoMetrix 0810164CF	3.6E+05 $TCID_{50}$/mL	None
Human Metapneumovirus B1	ZeptoMetrix 0810156CF	1.6E+04 $TCID_{50}$/mL	None
Human Metapneumovirus B2	ZeptoMetrix 0810162CF	1.3E+06 $TCID_{50}$/mL	None
Human rhinovirus A1	ZeptoMetrix 0810012CFN	1.3E+06 $TCID_{50}$/mL	None
Influenza A H1N1pdm09	ZeptoMetrix 0810538CF	1.4E+05 $TCID_{50}$/mL	None
Influenza A H3N2	ZeptoMetrix 0810526CF	7.2E+05 $TCID_{50}$/mL	None
Influenza B (Victoria Lineage)	BEI NR-44023	2.8E+08 $CEID_{50}$/mL	None
	ZeptoMetrix 0810037CF	2.5E+05 $TCID_{50}$/mL	None
Influenza B (Yamagata Lineage)	ZeptoMetrix 0810256CF	2.1E+04 $TCID_{50}$/mL	None
Parainfluenza virus 1	ZeptoMetrix 0810014CF	4.2E+05 $TCID_{50}$/mL	None
Parainfluenza virus 2	ZeptoMetrix 0810015CF	1.2E+07 $TCID_{50}$/mL	None
Parainfluenza virus 3	ZeptoMetrix 0810016CF	3.4E+07 $TCID_{50}$/mL	None
Parainfluenza virus 4	ZeptoMetrix 0810060CF	3.4E+07 $TCID_{50}$/mL	None
Respiratory syncytial virus A	ZeptoMetrix 0810040ACF	4.2E+05 $TCID_{50}$/mL	None
Respiratory syncytial virus B	ZeptoMetrix 0810479CF	4.2E+05 $TCID_{50}$/mL	None

[a] The HRV/EV assay may amplify off-target sequences found in strains of *Bordetella* species (*B. pertussis*, *B. parapertussis,* and *B. bronchiseptica*) when present at a concentration ≥1.3E+10 CFU/mL.

Table 23. Summary of Results for Off-Panel Organisms Tested During Evaluation of Analytical Specificity of the SPOTFIRE R Panel

Organism	Isolate ID	Concentration Tested	Observed Cross-Reactivity
Bacteria			
Arcanobacterium bernardiae	ATCC BAA-441	1.6E+09 cells/mL	None
Arcanobacterium haemolyticum	ATCC 9345	1.5E+08 cells/mL	None
Arcanobacterium pyogenes	ATCC 49698	6.7E+09 cells/mL	None
Bacillus cereus	ATCC 7064	8.3E+09 cells/mL	None
Bordetella bronchiseptica	ATCC 10580	8.3E+09 cells/mL	None
	ATCC 4617	7.9E+09 cells/mL	None
	ATCC 19395	7.9E+09 cells/mL	None
	NRRL B-59914	7.1E+09 cells/mL	None
	NRRL B-59909[a]	2.8E+01 cells/mL[b]	***Bordetella parapertussis***[a]
		2.8E+00 cells/mL[b]	None
Bordetella holmesii	ATCC 700052	8.3E+09 cells/mL	None
Burkholderia cepacia	ATCC 51671	7.9E+09 cells/mL	None
Campylobacter rectus	ATCC 33238	7.6E+07 cells/mL	None
Chlamydia trachomatis	ZeptoMetrix 0801775	1.3E+08 IFU/mL	None
Corynebacterium diptheriae	ATCC 27010	8.0E+09 cells/mL	None
Corynebacterium pseudodiphtheriticum	ATCC 10700	8.7E+09 cells/mL	None
Enterococcus casseliflavus	ATCC 49605	8.0E+09 cells/mL	None
Enterococcus faecalis	ZeptoMetrix 0801637	8.0E+09 CFU/mL	None
Escherichia coli	ATCC BAA-2196	7.2E+09 cells/mL	None
Fusobacterium necrophorum ssp. *funduliforme*	ATCC 51357	4.4E+08 cells/mL	None
Fusobacterium nucleatum	ATCC 25586	4.9E+08 cells/mL	None
Fusobacterium varium	ATCC 27725	1.6E+08 cells/mL	None
Gemella haemolysans	ATCC 10379	4.0E+09 cells/mL	None
Gemella morbillorum	ATCC 27824	1.0E+08 cells/mL	None
Granulicatella adiacens	ATCC 49175	1.3E+09 cells/mL	None
Haemophilus influenzae	ATCC 10211	8.3E+09 cells/mL	None
Haemophilus parahaemolyticus	ATCC 49700	8.7E+09 cells/mL	None
Klebsiella pneumoniae	CDC AR#0115	7.3E+09 CFU/mL	None

Organism	Isolate ID	Concentration Tested	Observed Cross-Reactivity
Lactobacillus rhamnosus	ATCC 7469	7.9E+09 cells/mL	None
Lactococcus lactis	ATCC 29146	6.2E+09 cells/mL	None
Legionella pneumophila	ATCC 33215	7.0E+09 cells/mL	None
Leptotrichia buccalis	ATCC 14201	4.4E+08 cells/mL	None
Moraxella catarrhalis	ATCC 43627	7.2E+09 cells/mL	None
Mycobacterium tuberculosis	ZeptoMetrix 0801660	6.1E+06 CFU/mL	None
Mycoplasma buccale	Mycoplasma Experience NC10136	1.4E+07 CFU/mL	None
Mycoplasma faucium	Mycoplasma Experience NC10174	1.4E+06 CFU/mL	None
Mycoplasma fermentans	Mycoplasma Experience NC10117	2.8E+07 CFU/mL	None
Mycoplasma genitalium	Mycoplasma Experience NC10195	1.8E+06 CFU/mL	None
Mycoplasma hominis	Mycoplasma Experience NC10111	1.2E+07 CFU/ml	None
Mycoplasma lipophilum	Mycoplasma Experience NC10173	1.5E+06 CFU/mL	None
Mycoplasma orale	Mycoplasma Experience NC10112	2.2E+07 CFU/mL	None
Mycoplasma salivarium	Mycoplasma Experience NC10113	4.4E+06 CFU/mL	None
Neisseria elongata	ATCC 25295	8.5E+09 cells/mL	None
Neisseria gonorrhoeae	ZeptoMetrix 0801482	4.9E+07 CFU/mL	None
Neisseria lactamica	ATCC 23971	2.7E+09 cells/mL	None
Neisseria meningitidis	ATCC 13113	7.4E+09 cells/mL	None
Neisseria sicca	ATCC 9913	7.2E+09 cells/mL	None
Neisseria subflava	ATCC 49275	8.0E+09 cells/mL	None
Parvimonas micra[c]	ATCC 33270	6.0E+07 cells/mL	None
Pneumocystis carinii	ATCC PRA-159	1.0E+07 nuclei/mL	None
Porphyromonas endodontalis	ATCC 35406	1.6E+07 cells/mL	None
Porphyromonas gingivalis	ATCC BAA-308	5.0E+08 cells/mL	None
Prevotella histicola	BEI HM-471	9.0E+08 cell/mL	None
Prevotella melaninogenica	ATCC 25845	6.9E+08 cells/mL	None
Prevotella oralis	ATCC 33322	6.2E+08 cells/mL	None
Pseudomonas aeruginosa	CDC AR#0092	8.3E+09 cells/mL	None
Rhodococcus equi	ATCC 33706	7.3E+09 cells/mL	None
Serratia marcescens	ATCC 27137	8.9E+09 cells/mL	None
Staphylococcus aureus	ATCC BAA-1700	7.4E+09 cells/mL	None
Staphylococcus epidermidis	ATCC 12228	8.0E+09 cells/mL	None
Staphylococcus haemolyticus	ATCC 29968	8.0E+09 cells/mL	None
Staphylococcus intermedius	ATCC 29663	8.2E+09 cells/mL	None
Staphylococcus saprophyticus	ATCC 15305	8.1E+09 cells/mL	None
Streptococcus agalactiae	ATCC 13813	6.0E+09 cells/mL	None
Streptococcus anginosus	ATCC 700231	7.1E+09 cells/mL	None
Streptococcus constellatus ssp. *pharyngis*	NCTC 13122	5.6E+08 cells/mL	None
Streptococcus dysgalactiae ssp. *dysgalactiae*	ATCC 43078	6.7E+09 cells/mL	None
	NCTC 4669	7.4E+09 cells/mL	None
	NCTC 4335	8.4E+09 cells/mL	None
	NCTC 4670	6.6E+09 cells/mL	None
	CCUG 27665	7.4E+09 cells/mL	None
	CCUG 28112	6.7E+09 cells/mL	None
	CCUG 28114	7.5E+09 cells/mL	None
Streptococcus dysgalactiae ssp. *equisimilis* (isolated from human)	ZeptoMetrix 0801516	7.8E+08 CFU/mL	None
Streptococcus dysgalactiae ssp. *equisimilis* (isolated from pig)	CCUG 28117	7.1E+09 cells/mL	None
Streptococcus dysgalactiae ssp. *equisimilis* (isolated from horse)	CCUG 27664	7.5E+09 cells/mL	None
	ATCC 10009	6.9E+09 cells/mL	None
Streptococcus gallolyticus	ATCC 43143	2.8E+09 cells/mL	None
Streptococcus gordonii	ATCC 10558	4.5E+09 cells/mL	None
Streptococcus intermedius	ATCC 27335	2.9E+09 cells/mL	None
Streptococcus mitis	ATCC 15914	3.2E+09 cells/mL	None
Streptococcus mutans	ATCC 25175	2.3E+09 cells/mL	None
Streptococcus oralis	ATCC 10557	1.1E+09 cells/mL	None
Streptococcus parasanguinis	ATCC 15912	7.8E+09 cells/mL	None
Streptococcus pneumoniae	ATCC 49619	2.5E+08 cells/mL	None
Streptococcus pyogenes	ATCC 12344	3.4E+08 cells/mL	None
Streptococcus salivarius	ATCC 13419	6.6E+09 cells/mL	None
Streptococcus sanguinis	ATCC 10556	1.1E+09 cells/mL	None
Tannerella forsythia	ATCC BAA-2717	2.6E+08 cells/mL	None

Organism	Isolate ID	Concentration Tested	Observed Cross-Reactivity
Treponema denticola	ATCC 33520	2.2E+08 cells/mL	None
Ureaplasma urealyticum	ATCC 27618	5.7E+07 cells/mL	None
Veillonella parvula	ATCC 10790	4.7E+08 cells/mL	None
Fungi			
Candida albicans	ATCC MYA-2876	2.8E+08 cells/mL	None
Saccharomyces cerevisiae	ATCC 18824	1.9E+08 cells/mL	None
Viruses			
Cytomegalovirus	ZeptoMetrix 0810003CF	1.9E+05 $TCID_{50}$/mL	None
Epstein-Barr virus	ZeptoMetrix 0810008CF	5.9E+06 copies/mL	None
Human herpes simplex virus 1	ATCC VR-260	8.9E+06 $TCID_{50}$/mL	None
Measles virus	ZeptoMetrix 0810025CF	2.5E+05 $TCID_{50}$/mL	None
Middle East respiratory syndrome coronavirus (heat-inactivated)	ZeptoMetrix 0810575CFHI	1.2E+05 $TCID_{50}$/mL	None
Mumps virus	ZeptoMetrix 0810079CF	2.0E+06 $TCID_{50}$/mL	None
Severe acute respiratory syndrome coronavirus (purified genomic RNA)	BEI NR-52346	5.3E+05 genomes/mL	None

[a] *Bordetella bronchiseptica* strain NRRL B-59909 contains genomic insertions of the IS*1001* gene and is reactive with the SPOTFIRE R Panel *Bordetella parapertussis* IS1001 assay .
[b] Positive results were observed for *Bordetella parapertussis* in all replicates tested at concentrations ≥2.8E+01 cells/mL and one out of three replicates tested at 2.8E+00 cells/mL.
[c] *Parvimonas micra* was formerly classified as *Micromonas micros* and *Peptostreptococcus micros*.

Near-LoD/Reproducibility Evaluation

A near-LoD/reproducibility evaluation was performed to demonstrate that the SPOTFIRE R Panel could reproducibly provide accurate results for weak-positive and negative samples when used by minimally trained operators. Contrived samples were tested at three of the prospective clinical study sites and additionally on three unique SPOTFIRE Systems at BioFire Diagnostics (BioFire) by trained BioFire personnel. The contrived samples contained combinations of SPOTFIRE R Panel analytes prepared in artificial matrix at or near (1× to 3×) the LoD. For testing performed at clinical sites, samples were tested over five testing events (non-consecutive days) by two operators during the course of their normal workday routine. Each site was equipped with a single SPOTFIRE System. Testing at all three sites was performed with a single reagent lot. For each testing event, each operator ran two replicate pouches for a total of 20 replicates per site and 60 total replicates across all three sites. For testing performed at BioFire, samples were tested over five consecutive days, by two operators per system, using three different reagent lots. Each day of testing, the two operators each tested three replicates on each system for a total of 30 replicates per system and 90 total replicates across all systems. When combined, each analyte was tested in a total of 150 replicates by at least 12 different operators across six different SPOTFIRE Systems.

A summary of results (percent (%) agreement with the expected positive or negative result) for each analyte (by site and system) is provided in Table 24. The SPOTFIRE R Panel reported the expected positive results for panel analytes in 94% -100% of samples and the expected negative results for all analytes in 100% of samples. Comparison of the positive percent agreement between user groups (98.8% for trained operators at BioFire versus 98.9% for minimally trained operators) demonstrates that the accuracy of the SPOTFIRE R Panel is not dependent upon the specific expertise of the user.

Table 24. Reproducibility of Results on the SPOTFIRE R Panel and BIOFIRE SPOTFIRE System

Analyte Isolate (Source ID)		**Concentration Tested (test level)**	**Expected Result**	**SpotFire System testing**								**All Sites /Systems** [95% Confidence Interval]
				BioFire Dx				**Clinical**				
				System A	**System B**	**System C**	**Total**	**Site 1**	**Site 2**	**Site 3**	**Total**	
Adenovirus Species B (ZeptoMetrix 0810062CF)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600 100%** [99.4-100%]
		2.4E+00 $TCID_{50}$/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**150/150 100%** [97.6-100%]
Bordetella parapertussis (ZeptoMetrix 0801462)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600 100%** [99.4-100%]
		1.2E+02 CFU/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**150/150 100%** [97.6-100%]
Bordetella pertussis (ZeptoMetrix 0801459)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600 100%** [99.4-100%]
		9.9E+02 CFU/mL (3× LoD)	**Positive**	29/30 (96.7%)	29/30 (96.7%)	30/30 (100%)	**88/90 (97.8%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**148/150 98.7%** [95.3-99.8%]
Chlamydia pneumoniae (ATCC 53592)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600 100%** [99.4-100%]
		2.0E+01 IFU/mL (1× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**150/150 100%** [97.6-100%]
Coronavirus (seasonal)	**No Analyte**		**Negative**	60/60 (100%)	60/60 (100%)	60/60 (100%)	180/180 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	**300/300 100%** [98.8-100%]
	229E (ATCC VR-740)	2.0E+00 $TCID_{50}$/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	**89/90 (98.9%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**149/150 99.3%** [96.3-100%]
	OC43 (ZeptoMetrix 0810024CF)	1.6E-02 $TCID_{50}$/mL (1× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**150/150 100%** [97.6-100%]
	NL63 (ZeptoMetrix 0810228CF)	2.5E-03 $TCID_{50}$/mL (1× LoD)	**Positive**	27/30 (90.0%)	30/30 (100%)	30/30 (100%)	**87/90 (96.7%)**	18/20 (90.0%)	20/20 (100%)	18/20 (90.0%)	**56/60 (93.3%)**	**143/150 95.3%** [90.6-98.1%]
Coronavirus SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2 (ATCC VR-1986HK)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600 100%** [99.4-100%]
		2.5E+02 copies/mL (1× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**150/150 100%** [97.6-100%]

Analyte Isolate (Source ID)	Concentration Tested (test level)	Expected Result	SpotFire System testing								All Sites /Systems [95% Confidence Interval]
			BioFire Dx				Clinical				
			System A	System B	System C	Total	Site 1	Site 2	Site 3	Total	
Human metapneumovirus 3 Type B1 (ZeptoMetrix 0810156CF)	**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600** **100%** [99.4-100%]
	7.5E-01 $TCID_{50}$/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90** **(100%)**	19/20 (95.0%)	20/20 (100%)	19/20 (95.0%)	**58/60** **(96.7%)**	**148/150** **98.7%** [95.3-99.8%]
Human rhinovirus/ enterovirus Enterovirus D68 US/MO/14-18947 (ATCC VR-1823)	**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600** **100%** [99.4-100%]
	1.1E+01 $TCID_{50}$/mL (1× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90** **(100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60** **(100%)**	**150/150** **100%** [97.6-100%]
Influenza A virus	**No Analyte**	**Negative**	90/90 (100%)	90/90 (100%)	90/90 (100%)	270/270 (100%)	60/60 (100%)	60/60 (100%)	60/60 (100%)	180/180 (100%)	**450/450** **100%** [99.2-100%]
	Influenza A H1N1pdm (ZeptoMetrix 0810538CF)	2.5E+00 $TCID_{50}$/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90** **(100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60** **(100%)**	**150/150** **100%** [97.6-100%]
	Influenza A H3N2 (ZeptoMetrix 0810526CF)	2.6E+00 $TCID_{50}$/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90** **(100%)**	19/20 (95.0%)	19/20 (95.0%)	20/20 (100%)	**58/60** **(96.7%)**	**148/150** **98.7%** [95.3-99.8%]
Influenza A virus A/H1-2009 Influenza A H1N1pdm (ZeptoMetrix 0810538CF)	**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600** **100%** [99.4-100%]
	2.5E+00 $TCID_{50}$/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90** **(100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60** **(100%)**	**150/150** **100%** [97.6-100%]
Influenza A virus A/H3 Influenza A H3N2 (ZeptoMetrix 0810526CF)	**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600** **100%** [99.4-100%]
	2.6E+00 $TCID_{50}$/mL (3× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90** **(100%)**	19/20 (95.0%)	19/20 (95.0%)	20/20 (100%)	**58/60** **(96.7%)**	**148/150** **98.7%** [95.3-99.8%]
Influenza B virus (ZeptoMetrix 0810037CF)	**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600** **100%** [99.4-100%]
	9.9E-02 $TCID_{50}$/mL (3× LoD)	**Positive**	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	**89/90** **(98.9%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60** **(100%)**	**149/150** **99.3%** [96.3-100%]
Mycoplasma pneumoniae (ZeptoMetrix 0801579)	**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600** **100%** [99.4-100%]

Analyte Isolate (Source ID)		Concentration Tested (test level)	Expected Result	SpotFire System testing								All Sites /Systems [95% Confidence Interval]
				BioFire Dx				Clinical				
				System A	System B	System C	Total	Site 1	Site 2	Site 3	Total	
		1.0E+01 CCU/mL (1× LoD)	**Positive**	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	**89/90 (99.3%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**149/150 99.3%** [96.3-100%]
Parainfluenza virus	**No Analyte**		**Negative**	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)	20/20 (100%)	20/20 (100%)	20/20 (100%)	60/60 (100%)	**150/150 100%** [97.6 100%]
	Parainfluenza virus 1 (ZeptoMetrix 0810014CF)	4.6E+00 $TCID_{50}$/mL (1× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**150/150 100%** [97.6-100%]
	Parainfluenza virus 2 (ZeptoMetrix 0810015CF)	4.2E+01 $TCID_{50}$/mL (3× LoD)	**Positive**	30/30 (100%)	29/30 (96.7%)	28/30 (93.3%)	**87/90 (96.7%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**147/150 98.0%** [94.3-99.6%]
	Parainfluenza virus 3 (ZeptoMetrix 0810016CF)	8.8E+00 $TCID_{50}$/mL (1× LoD)	**Positive**	28/30 (93.3%)	30/30 (100%)	30/30 (100%)	**88/90 (97.8%)**	19/20 (95.0%)	18/20 (90.0%)	20/20 (100%)	**57/60 (95.0%)**	**145/150 96.7%** [92.4-98.9%]
	Parainfluenza virus 4 (ZeptoMetrix 0810060CF)	2.0E+02 $TCID_{50}$/mL (1× LoD)	**Positive**	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	**89/90 (98.9%)**	20/20 (100%)	20/20 (100%)	20/20 (100%)	**60/60 (100%)**	**149/150 99.3%** [96.3-100%]
Respiratory syncytial virus (ZeptoMetrix 0810040ACF)		**No Analyte**	**Negative**	120/120 (100%)	120/120 (100%)	120/120 (100%)	360/360 (100%)	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (100%)	**600/600 100%** [99.4-100%]
		6.2E-02 $TCID_{50}$/mL (1× LoD)	**Positive**	30/30 (100%)	30/30 (100%)	30/30 (100%)	**90/90 (100%)**	20/20 (100%)	19/20 (95.0%)	20/20 (100%)	**59/60 (98.3%)**	**149/150 99.3%** [96.3-100%]
Total positive agreement (%) by system/user group				621/630 98.6%	628/630 99.7%	627/630 99.5%	**1876/1890 99.3%**	414/420 98.6%	415/420 98.8%	417/420 99.3%	**1246/1260 98.9%**	
Overall positive agreement (%) [95% Confidence Interval]												**3122/3150 99.1%** [98.7-99.4%]

Interference

Potentially interfering substances that could be present in NPS specimens or may be introduced into specimens during collection or subsequent handling and testing were evaluated for their effect on SPOTFIRE R Panel performance. The substances tested included endogenous substances that may be found at normal or elevated levels in clinical specimens (e.g. blood, mucus/mucin, human genomic DNA), various commensal or infectious microorganisms, medications, washes or topical applications for the nasal passage, various swabs and transport media for specimen collection, and substances used to clean, decontaminate, or disinfect work areas.

Each substance was added to contrived samples containing representative organisms at concentrations near (3×) the LoD. The concentration of substance added to the samples was equal to or greater than the highest level expected to be in NPS specimens.

Valid and accurate results were obtained for each sample containing substances and microorganisms at the concentrations listed in Table 25.

Table 25. Substances Tested on the SPOTFIRE R Panel - No Interference Observed

Substance Tested	Concentration Tested
Endogenous Substances	
Human Whole Blood (with Na Citrate)	10% (v/v)
Human Sputum/Mucus	1% (v/v)
Human Genomic DNA	20 ng/µL
Exogenous Substances[a]	
Promethazine hydrochloride	1.04 µmol/L (3.34E-04 mg/mL)
Acetaminophen (paracetamol)	1.0E+03 µmol/L (1.5E-01 mg/mL)
Acetylsalicylic acid (Aspirin)	167 µmol/L (3.0E-02 mg/mL)
Ibuprofen	1060 µmol/L (2.2E-01 mg/mL)
Albuterol sulfate (common ingredient in rescue inhalers)	0.188 µmol/L (5.4E-05 mg/mL)
Triple antibiotic ointment (neomycin/polymyxin B/bacitracin)	2% w/v
Mucinex® Severe Nasal Congestion Relief Clear & Cool Nasal Spray (Oxymetazoline hydrochloride 0.05%)	1% v/v
Saline nasal spray (sodium chloride 0.65%, disodium phosphate, phenylcarbinol, monosodium phosphate and benzalkonium chloride solution)	1% v/v
Vicks® VapoRub® Cough Suppressant Topical Analgesic (Camphor 4.8%, eucalyptus oil 1.2%, and menthol 2.6%)	1% w/v
Vaseline® Petroleum Jelly (100% white petrolatum)	1% w/v
Orajel™ (benzalkonium chloride 0.13%, benzocaine 20%, menthol 0.5%, zinc chloride 0.15%)	2% w/v
Chloraseptic® Sore Throat Spray (Phenol 1.4%)	1% v/v
Vicks® Formula 44™ DM (dextromethorphan hydrobromide 0.67 mg/mL, guaifenesin 13 mg/mL) (cough syrup)	1% v/v
Phenylephrine hydrochloride (common ingredient in nasal decongestants)	1% w/v
Nasal spray (fluticasone propionate 50 mcg)	1% v/v
Sucrets® Sore Throat (dyclonine hydrochloride 2.0 mg/lozenge)	1% w/v
Benadryl® Allergy Liqui-gels® (diphenhydramine hydrochloride 25 mg/capsule)	1% v/v
Zicam® Cold Remedy (Galphimia Glauca 4x, Luffa Operculata 4x, Sabadilla 4x)	1% v/v
Cold-eeze® (zinc gluconate 2.3%)	1% w/v
HALLS lozenge (menthol 5 mg/lozenge)	1% w/v
Listerine® Cool Mint® (menthol 0.042%, thymol 0.064%, methyl salicylate 0.06%, eucalyptol 0.092%)	6.5% v/v
Copenhagen® Snuff (Tobacco)	1% w/v
JUICE HEAD (30% propylene glycol, 70% vegetable glycerin) (e-juice)	1% v/v
Technique-Specific Substances	
Rayon swab (COPAN Diagnostics Inc.)	1 swab
Nylon flocked swab (COPAN Diagnostics Inc.)	1 swab
Polyester swab (COPAN Diagnostics Inc.)	1 swab
Calcium Alginate swab (Puritan®)	1 swab
Cary-Blair	90% v/v
Dulbecco's Modified Eagles-Medium (DMEM)	90% v/v
Hanks Balanced Salt Solution	100% v/v
0.9% Normal Saline	100% v/v
BD™ Universal Viral Transport	100% v/v
Remel MicroTest™ M4RT Tube w/o beads	100% v/v
Remel MicroTest™ M4 Tube w/o beads	90% v/v
Viral Preservative Media (VPM)	90% v/v
Phosphate Buffered Saline (PBS)	90% v/v
PrimeStore® MTM Molecular Transport Media	90% v/v
Stuart Transport Medium	90% v/v
eNAT™ Molecular Transport Medium	90% v/v
Bleach	1% v/v, 2% v/v[b]

Substance Tested	Concentration Tested
Ethanol	7% v/v
Disinfecting wipes (ammonium chloride)	0.25 – 0.5 inch square/sample
DNAZap™	1% v/v
RNaseZap™	1% v/v
Competing Microorganisms	
On-Panel	
Coronavirus 229E	1.5E+07 copies/mL
Enterovirus D68	7.8 E+07 copies/mL
Parainfluenza virus 3	8.0E+06 copies/mL
Respiratory syncytial virus A	1.5E+07 copies/mL
Adenovirus A31	1.6E+07 copies/mL
Bordetella pertussis	1.6E+09 copies/mL
Off-Panel	
Cytomegalovirus (CMV)	4.2E+04 $TCID_{50}$/mL
Herpes simplex virus 1	9.0E+06 $TCID_{50}$/mL
Staphylococcus aureus	7.4E+08 CFU/mL
Streptococcus pneumoniae	2.5E+07 CFU/mL
Streptococcus pyogenes	2.2E+08 copies/mL
Haemophilus influenzae	8.3E+08 CFU/mL
Candida albicans	2.8E+07 CFU/mL

[a] Nasal influenza vaccines (e.g. FluMist®) were not evaluated but are predicted to be reactive with the Influenza A (subtype) and Influenza B assays.
[b] Incubation of sample with 1% (v/v) bleach for 15 minutes, 4 hours, or ~18.5-hour (overnight) or 2% (v/v) bleach for 15 minutes did not result in interference.

NOTE: Avoid contact between samples and bleach prior to testing (bleach can damage nucleic acids and prevent amplification and detection by the panel).

NOTE: Compatibility of the SPOTFIRE R Panel with NPS in PrimeStore® MTM has not been evaluated in the intended use setting. PrimeStore® MTM and BIOFIRE Sample Buffer contain guanidine salts that will react with bleach to form a toxic gas. Use caution if using bleach for disinfection purposes when collecting or testing NPS specimens.

External Control Material

Quality Control Testing

External control material is available directly from bioMérieux and Maine Molecular Quality Controls, Inc. (MMQCI) (online at www.mmqci.com). The MMQCI controls listed in Table 26 are positive and negative control solutions for all viruses and bacteria detected by the SPOTFIRE R Panel, along with an insert explaining the expected results.

Table 26. Control Materials Compatible with all Analytes on the SPOTFIRE R Panel

Vendor	Product Name	Part Number
Maine Molecular Quality Controls, Inc. (MMQCI)	SPOTFIRE® RSP Negative Control	M42738
	SPOTFIRE® RSP Positive Control	M42638

NOTE: MMQCI control test solutions can be stored at room temperature (18°C-25°C).

It is recommended that when using the SPOTFIRE R Panel that Positive and Negative QC testing should be performed when:

- Training a new operator
- Receiving a new shipment or lot of SPOTFIRE R Panel test kits
- Receiving a new SpotFire Control Station

A Negative QC test should be run at least monthly for each testing location to monitor for environmental contamination. Additional Negative QC tests may be run if contamination is suspected.